8th International Mycological Congress

IMC8 2006 CAIRNS

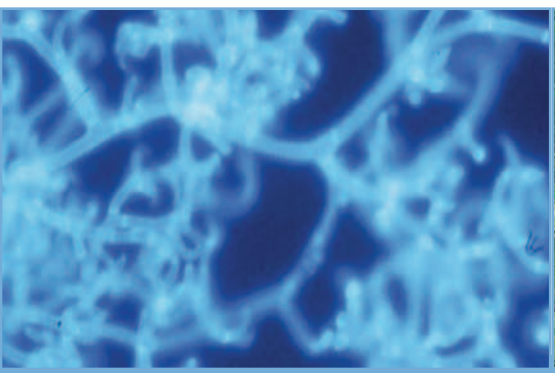
21 – 25 August, 2006

Cairns Convention Centre
Queensland, Australia

First in the Southern Hemisphere



Congress Handbook & Abstracts Book 2



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The Organising Committee of the 8th International Mycological Congress would like to sincerely thank the following sponsors and exhibitors for their generous support.

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MAF BIOSECURITY NEW ZEALAND

The Australian Society for Microbiology

MSA the Mycological society of america

bms British Mycological Society promoting fungal science

日本菌学会 The Mycological Society of Japan

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Cambridge University Press
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AUSTRALASIAN MYCOLOGICAL SOCIETY

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WELCOME TO IMC8 CAIRNS 2006

On behalf of the organising committee, it is our pleasure to welcome you to the first International Mycological Congress to be held in the southern hemisphere, IMC8 in Cairns, Australia.

It has been a challenge to design and bring to fruition a congress that can meet the high standards set by previous Congresses. We hope to provide you with a congress experience that is stimulating, rewarding and enjoyable.

We have developed an outstanding program, thanks to our national and international scientific program organising committee, and to your scientific input and participation. There are nine pre-congress workshops to choose from, and within the congress program, there is an invited internationally recognised plenary speaker each day. The daily program encompasses five concurrent sessions of scientific symposia that include a combination of invited speakers and presenters chosen from submitted abstracts, and an extensive range of scientific poster presentations for your perusal. We trust that there will be something of interest to all mycologists in our large and varied scientific program.

We invite you to attend the social events. On Monday evening, the Mycological Society of America and the British Mycological Society host a reception on the waterfront at the Cairns Hilton Hotel. Everyone is invited to attend.

Building on a previous successful IMC convention, please join us for a 'Wines of the World' evening on Tuesday night. You bring a bottle of wine to share with your friends and colleagues, and in an atmosphere of joviality, we challenge you to approach as many people as possible to taste and judge your wine. Prizes go to the best and worst wines of the world! This is a great networking experience/opportunity? and a great way to make new friends.

The Congress dinner will be held on Thursday evening at 'The Tanks'. This will be a unique Cairns dining experience, within the Botanic Gardens precinct.

During the Congress, 'The Clamp Connection Café/Bar' will be open to meet your 'between break' beverage and snack needs. Feel free to take advantage of this relaxed space to catch up with colleagues and friends, and to develop new research collaborations and partnerships.

A number of mycology-orientated tours and forays have been organised, and we encourage you to take the opportunity to explore Cairns and ints hinterland on these tours.

We promise you an outstanding scientific program and a memorable social experience in Australia. The Congress will be the biggest mycology meeting ever held in Australia. It will provide a forum for the sharing of information on all aspects of mycology and foster constructive interaction between participants from all over the world.

With its combined World Heritage-listed attractions of the Great Barrier Reef and the Wet Tropic Rainforests, Cairns has a lot to offer. We hope that you are able to take some time to visit the area and discover the natural wonders of tropical northern Australia.

Thank you for coming, we trust you find your visit enjoyable and rewarding.

Wieland Meyer
Chair
IMC8 Organizing Committee

Ceri Pearce
Vice-Chair

IMC8 2006 ORGANISING COMMITTEE

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Ceri Pearce, Vice-Chair
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GENERAL INFORMATION

The following information is offered to make your attendance at IMC8 2006 as pleasant and as trouble-free as possible. If you require help, please call at the registration Desk and we will do everything we can to assist you.

Registration and Information Desk
The Registration Desk will be located in the main foyer of the Cairns Convention Centre. It will be open at the following times:

Sunday 20th August	14:00-18:00
Monday 21st August	07:00-17:30
Tuesday 22nd August	07:30-17:30
Wednesday 23rd August	07:30-18:00
Thursday 24th August	07:30-17:30
Friday 25th August	07:30-18:00

Accommodation Contacts

Map ref		
B	Hilton Cairns	07 4050 2000
C	Sofitel Reef Casino Cairns	07 4060 8888
D	Holiday Inn Cairns	07 4050 6070
E	Oasis Resort Cairns	07 4080 1888
F	Pacific International Cairns	07 4051 7888
G	Tradewinds Esplanade Hotel	07 4053 0300
H	Heritage Cairns	07 4051 1211
	Rydges Esplanade Resort	07 4031 2211
I	Rydges Plaza Cairns	07 4046 0300
J	Club Crocodile Hides Hotel	07 4051 1266
K	Cairns Holiday Lodge	07 4051 4611
L	181 The Esplanade	07 4052 6888
M	Cairns Aquarius Apartments	07 4051 8444
O	Inn Cairns Boutique Apartments	07 4041 2350
	Mantra Trilogy Resort	07 4080 8000
P	Mid City Luxury Suites	07 4051 5050
	Oaks City Quays & Piermonde Cairns	07 4042 6400
Q	City Terraces Apartments	07 4051 8955
R	Il Centro Luxury Apartments	07 4031 6699
S	Il Palazzo Boutique Apartment	07 4041 2155
T	Southern Cross Atrium Apartments	07 4031 4000
U	Gilligan's Backpackers Hotel & Resort	07 4041 6566

Accreditation
A Certificate of Attendance will be provided indicating your attendance at the Congress. Individuals may then claim CPD points from their respective organisations.

Name Tags
For security purposes your name tag must be worn at all times. Entry to all sessions, exhibition and Welcome Reception is by name tag only.

Banking
Normal banking hours are Monday - Thursday 9:30am-4:00pm and Fridays 9:30-5:00pm, excluding public holidays. 24 Hour Automatic Teller Machines (ATMs) can be found throughout the City and Shopping Centres and in the Sofitel Reef Casino Cairns.

Coat Check/Bag Store
The Cairns Convention Centre does not have a facility. When checking out of your hotel for your return journey home, please make arrangements for your bags to be stored at the hotel.

Childcare
Please note, no official arrangements have been made for childcare during the congress. Your chosen accommodation may be able to assist you further with babysitting services during your stay.

Public Internet Facilities
Inbox Cafe- 119 Abbott St, Cairns
Reff Highspeed Netcafe- shp 2/31 Shields st, Cairns
The Call Station- 123 Abbott st, Cairns
Gilligan's - Grafton St
Global Gossip Cairns- 125 Abbott st, Cairns
Wireless facilities are available at the Convention Centre

Goods and Services Tax (GST) / Tourist Refund Scheme (TRS)
GST is included in all prices, unless otherwise stated. You can claim a refund of the GST and wine equalisation tax (WEX) that you pay on goods you buy in Australia. The refund only applies to goods you take with you as hand luggage or wear on to the aircraft when you leave the country. (the goods can be used in Australia before departure). To qualify for the TRS, you must: spend $300 or more in the one store and get a single tax invoice; buy goods no more than 30 days before departure; wear or carry the goods on board and present them along with your tax invoice, passport and boarding pass to a TRS facility. *Claims are only available up to 30 minutes prior to the scheduled departure of your flight.*

Medical Services
If you require medical assistance, please contact the Registration Desk staff.

Messages
Message sent to the secretariat will be placed on a notice board near the Registration Desk. The secretariat will not locate the individual delegate.

Mobile Phones
Please respect the presenter and other members of the audience by ensuring your mobile phone is switched off or to silent while you are in sessions.

Parking
Cairns Convention Centre has a public Car park below. Entry is via Sheridan Street. $3 coin operated boomgate. Carpark is open from 7am-until program concludes.
Parking metres are around the CBD area. You have to pay from approx 8.30am - 5.00pm.
In the pier marketplace carpark, which is located on Esplanade, you still have to pay until 10pm. Cairns Central Shopping Centre is Free of Charge + along wharf St, you can park your car for $2 per day or undercover at the Casino for $5 per day.

Shopping
There is a shopping facilities within a 5 min walk of the Cairns Convention Centre: either Cairns Central Shopping Centre on McLeod Street or the Mall located on Cnr Shields & Lake Street. General Shops i.e Cairns Central are open approx 9.30am - 5.30pm
Supermarkets are open to approx 9pm on weekdays and 5.30pm weekends.

Post Offices
Orchard Plaza - Abbott St
Cairns Central Shopping Centre
Grafton St (2 min walk)

Pharmacies
Cairns Central Chemart Pharmacy
Terry White Cairns Central Shopping Centre
Pharmacy - Shop 1/86 Lake St, Cairns
Chemist Warehouse - 50 McLeod St, Cairns
Esplanade Day & Night Pharmacy - Shop 10/85 The Esplanade

Refreshments
Morning tea and afternoon tea are included in your registration fee and are provided during the programmed breaks in the exhibition and outside patio areas.

Lunches: are not included in the registration. Tickets can be bought for lunches at AU$15.00 per person per day, order by 5pm the day prior. Pre purchase was required and your tickets will be in your registration pack.

Supper Meals: a light Meal will be available on a ticket basis AU$22.00 each, for Monday and Tuesday. Pre purchase was required and your tickets will be in your registration pack.

Clamp Connection Café/Bar:12 noon-close of conference each day! The Café offers you an opportunity to spend time talking with representatives from exhibition stands, viewing poster displays, and networking with colleagues and new friends. Refreshment, beer, wine, soft drinks, tea and coffee will be available on a cash basis.

Speakers Preparation Room – Meeting Room 9
All Presenters must check in to ensure your audio visual needs are confirmed, and also that you have arrived at the congress in time to present.

Special Dietary Requirements
Delegates who have specified their special dietary requirement on their registration form should identify themselves to the service staff at the Convention Centre.

Telephones
Public Telephones, coin operated, are located in the Sheridan St foyer.

Tipping
Tipping is not expected in Australia but is appreciated for particularly good service.

Telephone Directory

Emergency Services 000
(fire/police/ambulance)
Registration Desk
Tel: 07 4042 4300 Fax: 07 4042 4302
Taxi Services
Black and White Taxi - 13 10 08
Taxi phone is located outside the main entrance of the centre.
Airlines

Qantas	131 313
Virgin Blue	136 789
Jetstar	131 538
Air New Zealand	132476
Cathay Pacific	131747
Japan Airlines	(07)3229-9916

Travel and Touring
There will be a Visitor Information booth situated in Hall 2 to assist you with any tours and travel enquiries that you might have.

Posters
The Posters will be judged by a panel and prizes awarded.

Registration Cancellation & Refund Policy
Cancellations received on or before Friday 7 July 2006 received a refund of registration fees, less an administrative charge of AUD$100.00. Cancellations after this date are not refunded

SOCIAL PROGRAM

Welcome reception - Sunday 20 August -
Cairns Convention Centre - *1800–1930*
Renew old acquaintances and join fellow delegates for informal drinks and canapés in the exhibition area for IMC8.
Cost included in registration. Entry is via name tag or ticket.
Dress: Casual.

BMS/MSA joint Reception - Monday 21 August -
Cairns Hilton- *2000-2100*
The British Mycological Society and the Mycological Society of America are jointly hosting a reception following the Honorary Lecture.
Included in registration.
Dress: Casual. Delegates will make their own way to the venue.

Wines of the World - Wednesday 23 August -
Hall 2 – *1900-2200 hours*
Bring a bottle of wine along to the Convention Centre and share with your colleagues.
Tickets: AU$22 per person.
Dress: Casual. Delegates will make their own way to the venue.

Congress Dinner - Thursday 24 August – The Tanks -
1900–2400 hours
The premier social occasion of the congress will be an event not to be missed!. Not included in registration.
Tickets: AU$120 per person includes three course meal and beverages.
Dress: Smart casual. Delegates will be collected from their hotels from 1830 and shuttle busses will return guests from 2230.

Partners program
A Partners program has been developed as an introduction to Cairns and its attractions. Included in the program is the Welcome Reception on Sunday. On Monday the day will start with a morning tea at the Cairns Convention Centre at 0930 followed by a City Sights Tour and concluding with a lunch back at the Cairns Convention Centre.

Posters
The Posters will be judged by a panel and prizes awarded.

Registration Cancellation & Refund Policy
Cancellations received on or before Friday 7 July 2006 received a refund of registration fees, less an administrative charge of AUD$100.00. Cancellations after this date are not refunded.

Workshops and Tours

Wednesday - 16th – Friday 18th August

0900-1800 - Workshop 1 (by Invitation only): Ceratocystis and Ophistoma

Moreton Bay Research Station, Brisbane
Chair: Mike Wingfield (South Africa) / Keith Seifert (Canada)

Friday 18th August

0730-1800 - Cairns Hinterland Lichen Tour

Friday 18 August to Saturday 19 August 2006, and Sunday 20th August at James Cook University

0730-1800 - Daintree Rainforest Photo Tour

0900-1800 - Workshop 2: Filamentous Fungi in the Clinical Laboratory

James Cook University, Cairns
Chair: Richard Summerbell (The Netherlands)

0900-1800 - Workshop 3: Insect Pathogens in the Tropics

James Cook University
Chair: Nigel Hywel-Jones (Thailand)

Saturday 19th August

0730-1800 - Cairns Hinterland Lichen Tour cont…

Friday 18 August to Saturday 19 August 2006, and Sunday 20th August at James Cook University

0900-1800 - Workshop 4: Food Mycology and Mycotoxins

Cairns Convention Centre
Chairs: John Pitt (Australia) / Ailsa Hocking (Australia) / Brett Summerell (Australia)

0900-1800 - Workshop 5: Rust Taxonomy

James Cook University
Chair: Yoshitaka Ono (Japan)

0900-1800 - Workshop 9: Hypogeous Fungi

James Cook University
Chairs: Teresa Lebel (Australia) / Sandra Abell (Australia)

Sunday 20th August

0900-1700 - Cairns Hinterland Lichen Tour – details above

James Cook University
Pick up from your hotels and transfer to James Cook University.

0900-1800 - Workshop 4: Food Mycology and Mycotoxins cont…

Cairns Convention Centre
Chairs: John Pitt (Australia) / Ailsa Hocking (Australia) / Brett Summerell (Australia)

0900-1800 - Workshop 6: Smut Taxonomy Workshop

James Cook University
Chairs: Kálmán Vánky (Germany) / Roger Shivas (Australia)

0900-1800 - Workshop 7: AnaSat2: From Spore to Culture

James Cook University
Chairs: Pedro Crous (The Netherlands) / Keith Seifert (Canada)

0900-1800 - Workshop 8: Compendium of Rust Fungi

James Cook University
Chairs: Reinhard Berndt (Switzerland) / Yoshitaka Ono (Japan)

Monday 21st August 2006

Time	Activity				
07:00	**Registration** *Foyer*				
08:30	**Opening Ceremony** *Halls A & B*				
09:15	**Plenary 1:** Franz Oberwinkler (Germany) **Fungal Tree of Life** *Halls A & B*				
10:15	**Coffee Break** – *Hall 2*				
10:45	**Symposium 1** *Halls A & B* **Phylogenetic Biology of Fungal and Fungal-like Phyla** Rytas Vilgalys (USA) Joseph Spatafora (USA)	**Symposium 2** *Hall C* **Do Plant Pathogens have a specific Armory?** Barbara Howlett (Australia) Thierry Rouxel (France)	**Symposium 3** *MR 1 & 2* **Insect Associated Fungi** Diana Six (USA) Meredith Blackwell (USA)	**Symposium 4** *Hall D* **Fungal Cell Biology** Reinhard Fischer (Germany)	**Symposium 5** *MR 3 - 5* **Ecology and Diversity of *Penicillium* and *Aspergillus* in Australia** John Pitt (Australia) Ailsa Hocking (Australia)
12:45	**Lunch [pre purchase]** – *Hall 2*				
13:30	**Poster Session 1: Phylogeny, Systematics & Evolution** **Poster Session 9: Mycorrhizae**				
15:00	**Coffee Break** – *Hall 2*				
15:30	**Symposium 6** *MR 1 & 2* **Molecular Taxonomy of Yeast** Cletus Kurtzman (USA) Andre Lachance (Canada)	**Symposium 7** *Hall C* **Transcriptome Analyses of Fungal Pathogens during Infection** Yue Wang (Singapore) Marc-Henri Lebrun (France)	**Symposium 8** *MR 3 - 5* **Diversity of Microfungi in Neotropica** Jose Dianese (Brazil) José Hernandez (Argentina)	**Symposium 9** *Hall D* **The Fungal Septum and associated Organelles** Katsu Kitamoto (Japan) Gregory Jedd (Singapore)	**Symposium 10** *Halls A & B* **Population Genetics of Fungi** Jeremy Burdon (Australia) Linda Kohn (Canada)
17:30	**Poster Session / Supper [pre purchase] / "Clamp Connection Café/Bar"** – ***cash basis bar for drinks and coffee*** – *Hall 2*				
19:00	**Honorary Lecture:** David Hawksworth (Spain) **Mycology and Mycologists** *Halls A & B*				
20:00	**Joint Reception of the British Mycological Society and the Mycological Society of America** *Cairns Hilton*				

Tuesday 22nd August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	**Symposium 11** *Hall D* **Ascomycete Phylogenetics** Kevin Hyde (Hong Kong) Joseph Spatafora (USA)	**Symposium 12** *Halls A & B* **Proteome Analysis** Jim Kronstad (Canada) Scott E. Baker (USA)	**Symposium 13** *Hall C* **Mycorrhizal Ecology** Tom Bruns (USA) John Kilronomos (Canada)	**Symposium 14** *MR 1 & 2* **Propagation Strategies of Fungi** Akira Suzuki (Japan) Felix Baerlocher (Canada)	**Symposium 15** *MR 3 - 5* **Emerging and Reemerging Pathogenic Fungi** Hester Vismer (South Africa) Sue Coloe (Australia)
10:00	**Coffee Break** – *Hall 2*				
10:30	**Plenary 2:** John Taylor (USA) **Species Concepts in Fungi** *Halls A & B*				
11:30	**Lunch [pre purchase]** – *Hall 2*				
12:00	**Poster Session 2: From Genomics to Proteomics** **Poster Session 6: Food Mycology and Mycotoxins**				
13:30	**Symposium 16** *Halls A & B* **Fungal Phylogenomics** Teun Boekhout (Netherlands) Bernard Dujon (France)	**Symposium 17** *Hall C* **Signal Transduction during Pathogenesis** Jinh-Rong Xu (USA) Marty Dickman (USA)	**Symposium 18** *MR 3 - 5* **Veterinary Mycology** Kishio Hatai (Japan) Mark Krockenberger (Australia)	**Symposium 19** *Hall D* **Substrate Colonisation and Succession in Wood Inhabiting Fungi** Jan Stenlid (Sweden) Lynne Boddy (UK)	**Symposium 20** *MR 1 & 2* **Fungi of Monsoon Asia: - Linking Diversity and Ecosystem Function** Morten Christensen (Denmark) Seiji Tokumasu (Japan)
15:30	**Coffee Break** – *Hall 2*				
16.00	**Symposium 21** *Halls A & B* **DNA Barcoding for Fungi** Richard Summerbell (The Netherlands) Andre Levesque (Canada)	**Symposium 22** *MR 1 & 2* **Polyketides, Non-Ribosomal Peptides, and Terpenes as fungal signal molecules** Jens Frisvard (Denmark) Barry Scott (New Zealand)	**Symposium 23** *Hall C* **Adhesion of Fungi to Plant or Animal Hosts** Nick Talbot (UK) Ester Segal (Israel)	**Symposium 24** *MR 3 - 5* **Protein Secretion in Fungal Biotechnology** David Archer (UK) Merja Penttila (Finland)	**Symposium 25** *Hall D* **Evolution of Symbioses** Dominik Begerow (Germany) Martin Grube (Austria)
18:00	**Supper [pre purchase] / "Clamp Connection Café/Bar"** – *cash basis bar for drinks and coffee* – *Hall 2*				
18:30	**Poster Session** *Hall 2*			**Roundtable 1** *Halls A & B* **Is it Time for a Mycological Code of Nomenclature?** Pedro Crous, David Minter, Amy Rossman, Franz Oberwinkler, John Taylor, Tsuyoshi Hosoya	
20:00	**MSJ Editorial Meeting** *MR8*				
20:30	**Evening Free**				

Wednesday 23rd August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	**Discussion Group** *MR 1 & 2* **Lichen-Fungal Genome Sequencing Project** Paul Dyer (UK)	**Australasian Mycological Society AGM** *Hall C*	**Discussion Group** *MR 3 - 5* **Fungal Genome Sequencing Programs at the DOE Joint Genome Institute** Scott Baker (USA)		
09:30	**Break**				
10.00	**Plenary 3:** James Galagan (USA) **Comparative Fungal Genomics** *Halls A & B*				
11:00	**Coffee Break** – *Hall 2*				
11:30	**Symposium 26** *Halls A & B* **Lichen Symbiosis: Extraterrestrial Life, Evolution and Penguin Rookery** Francois Lutzoni (USA) Magdalena Pavlich (Peru)	**Symposium 57** *MR 3 - 5* **Chytridiomycete Fungi** Gordon Beakes (UK) Peter McGee (Australia)	**Symposium 28** *MR 1 & 2* **Enzymes and Infection Mechanisms** Michel Monod (Switzerland) Matt Templeton (New Zealand)	**Symposium 29** *Hall D* **Fungal Physiology** Helena Nevalainen (Australia) Ken Hammel (USA)	**Symposium 30** *Hal* **Asia-Pacific Fungal Biodiversity** Gareth Jones (Thailand) Sitti Aisyah Alias (Thailand)
13.30	**Lunch [pre purchase]** – *Hall 2*				
14.00	**Poster Session 3: Plant and Fungal Pathogens** **Poster Session 10: Animal Pathogens**				
15:00	**Coffee Break** – *Hall 2*				
15:30	**Symposium 31** *MR 3 - 5* **Systematics and Ecology of Dimorphic Basidiomycetes** Alvaro Fonseca (Portugal) Jose Paulo Sampaio (Portugal)	**Symposium 32** *MR 1 & 2* **Bioinformatics and Databases** Vincent Robert (Netherlands) Peter Dawyndt (Belgium)	**Symposium 33** *Hall D* **Antifungal Resistance** Richard Cannon (New Zealand) Dominique Sanglard (Switzerland)	**Symposium 34** *Hall C* **Importance of Small Non-Coding RNAs in Fungi** Carlo Cogoni (Italy) Rodolfo Aramayo (USA)	**Symposium 35** *Halls A* **Gondwanan Fungi** Tom May (Australia) Peter Buchanan (New Zealand)
17:30	**"Clamp Connection Café/Bar"** – *cash basis bar for drinks and coffee* – *Hall 2*				
18:00	**Poster Session** *Hall 2*	**Roundtable 2** *Hall C* **Access and benefit sharing in relation to the Biodiversity Convention** Lene Lange (Denmark)	17:45-19:00 **Meeting of the International Association Lichenology** *MR1&2*		
20:00	**Wines of the World** *Hall 2*				

Thursday 24th August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	**General Meeting of the International Commission for the Taxonomy of Fungi** *MR 1 & 2* Keith Seifert (Canada)	**Symposium 42** *Hall C* **Genomes and Fitness** Gareth Griffith (UK) Simon Avery (UK)	**Proffered Session 1** *MR 3 - 5* **Phylogeny 1** Heide-Marie Daniel (Belgium)	**Proffered Session 2** *Hall D* **Medical Mycology** Sharon Chen (Australia)	
10:00	**Break**				
10:15	**Plenary 4:** Regine Kahmann (Germany) **Mating in Fungi** *Halls A & B*				
11:15	**Coffee Break** – *Hall 2*				
11:45	**Symposium 36** *MR 1 & 2* **Straminopiles: Why and How?** Daiske Honda (Japan) Gordon Beakes (UK)	**Symposium 37** *MR 3 - 5* **Advanced Cellular Imaging and Micromanipulation** Nick Read (UK) Rosa Morinho-Perez (Mexico)	**Symposium 38** *Hall C* **Fungal Pigments and Virulence** Josh Nosanchuck (USA) Beatriz L. Gomez (UK)	**Symposium 39** *Halls A & B* **Biosynthetic Gene Clusters for Fungal Secondary Metabolites** Nancy Keller (USA) Marc Stadler (Germany)	**Symposium 40** *Hall D* **Biosecurity** Geoff Ridley (New Zealand) Ceri Pearce (Australia)
13:45	**Lunch [pre purchase]** – *Hall 2*				
14:30	**Poster Session 4: Cell Biology and Physiology** **Poster Session 8: Population Genetics**				
15:00	**Coffee Break** – *Hall 2*				
15:30	**Symposium 41** *MR 1 & 2* **Evolution, Ecology and Systematics of Endophytic Fungi - Horozontally Transmitted Endophytes** Elizabeth Arnold (USA) Gerard Verkley (The Netherlands)	**Symposium 56** *MR 3 - 5* **Phylogeography** Greg Mueller (USA) Thorsten Lumbsch (USA	**Symposium 43** *Hall D* **Biocontrol** Augusto Schrank (Brazil) Naresh Magan (UK)	**Symposium 44** *Hall C* **Industrial Mycology** Cees van den Hondel (Netherlands) Lene Lange (Denmark)	**Symposium 45** *Halls A & B* **Worldwide Movement of Fungal Forest Pathogens** Brenda Wingfield (South Africa) Matteo Garbelotto (USA)
17:30	**Poster Session / "Clamp Connection Café/Bar"** – *cash basis bar for drinks and coffee* – *Hall 2*				
19:00-23:30	**Conference Dinner** *The Tanks*				

Friday 25th August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	*Conference Room 1* **International Mycological Association (IMA) Board Meeting**	**Proffered Session 3** *Hall C* **Phylogeny 2** Clement Tsui (Australia)		**Proffered Session 4** *Hall D* **From Mycological Diversity to Phylogeny** Kálmán Vánky (Germany)	
10:00	**Coffee Break** – *Hall 2*				
10:30	**Symposium 46** *Hall C* **Anything Specific about Human Pathogens?** Alex Adrianopoulos (Australia) James Fraser (Australia)	**Symposium 47** *Halls A & B* **Biodiversity of Microfungi - A Phylogenetic Approach** Andrew Miller (USA) Amy Rossman (USA)	**Symposium 48** *Hall D* **Molecular Plant Mycorrhizal Interaction** Mark Tibbett (Australia) Paola Bonfante (Italy)	**Symposium 49** *MR 1 & 2* **Marine Fungi** Ka-Lai Pang (Hong Kong) Mohamed Abdel-Wahab (Egypt)	**Symposium 50** *MR 3 - 5* **Mycetozoan Biodiversity** Steven Stephenson (USA) David Orlovich (New Zealand)
12:30	**Lunch [pre purchase]** – *Hall 2*				
13:30	**Poster Session 5: Biodiversity and Conservation** **Poster Session 7: Industrial Mycology**				
14:30	**Symposium 51** *Hall D* **Finding the Missing Taxa: the Search for Fungi in Under-explored Habitats** Wendy Untereiner (Canada) James Scott (Canada)	**Symposium 52** *Hall C* **Fungi and Eucalypts** Eric McKenzie (New Zealand) David Ellis (Australia)	**Symposium 53** *MR 3 - 5* **Epidemiology of Fungal Pathogens** Wieland Meyer (Australia) Rosely Zancope-Olivera (Brazil)	**Symposium 54** *Halls A & B* **Fusarium - New Advances in Taxonomy, Biology and Detection** Brett Summerell (Australia) Keith Seifert (Canada)	**Symposium 55** *MR 1 & 2* **Conservation and Utilization of Fungal Biodiversity through Genetic Resource Centres** Pedro Crous (Netherlands) Akira Nakagiri (Japan)
16:30	**Coffee Break / "Clamp Connection" closes** – *Hall 2*				
17:00	**Plenary 5:** Mike Wingfield (South Africa) **Forest Fungi in a Changing World** *Halls A & B*				
18:00	**Closing Ceremony** *Halls A & B*				

Thursday 24th August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	**General Meeting of the International Commission for the Taxonomy of Fungi** *MR 1 & 2* Keith Seifert (Canada)	**Symposium 42** *Hall C* **Genomes and Fitness** Gareth Griffith (UK) Simon Avery (UK)	**Proffered Session 1** *MR 3 - 5* **Phylogeny 1** Heide-Marie Daniel (Belgium)	**Proffered Session 2** *Hall D* **Medical Mycology** Sharon Chen (Australia)	
10:00	**Break**				
10:15	**Plenary 4:** Regine Kahmann (Germany) **Mating in Fungi** *Halls A & B*				
11:15	**Coffee Break** – *Hall 2*				
11:45	**Symposium 36** *MR 1 & 2* **Straminopiles: Why and How?** Daiske Honda (Japan) Gordon Beakes (UK)	**Symposium 37** *MR 3 - 5* **Advanced Cellular Imaging and Micromanipulation** Nick Read (UK) Rosa Morinho-Perez (Mexico)	**Symposium 38** *Hall C* **Fungal Pigments and Virulence** Josh Nosanchuck (USA) Beatriz L. Gomez (UK)	**Symposium 39** *Halls A & B* **Biosynthetic Gene Clusters for Fungal Secondary Metabolites** Nancy Keller (USA) Marc Stadler (Germany)	**Symposium 40** *Hall D* **Biosecurity** Geoff Ridley (New Zealand) Ceri Pearce (Australia)
13:45	**Lunch [pre purchase]** – *Hall 2*				
14:30	**Poster Session 4: Cell Biology and Physiology** **Poster Session 8: Population Genetics**				
15:00	**Coffee Break** – *Hall 2*				
15:30	**Symposium 41** *MR 1 & 2* **Evolution, Ecology and Systematics of Endophytic Fungi - Horozontally Transmitted Endophytes** Elizabeth Arnold (USA) Gerard Verkley (The Netherlands)	**Symposium 56** *MR 3 - 5* **Phylogeography** Greg Mueller (USA) Thorsten Lumbsch (USA	**Symposium 43** *Hall D* **Biocontrol** Augusto Schrank (Brazil) Naresh Magan (UK)	**Symposium 44** *Hall C* **Industrial Mycology** Cees van den Hondel (Netherlands) Lene Lange (Denmark)	**Symposium 45** *Halls A & B* **Worldwide Movement of Fungal Forest Pathogens** Brenda Wingfield (South Africa) Matteo Garbelotto (USA)
17:30	**Poster Session / "Clamp Connection Café/Bar"** – ***cash basis bar for drinks and coffee*** – *Hall 2*				
19:00-23:30	**Conference Dinner** *The Tanks*				

0730-0930 **Meeting Room 1&2**

General Meeting of the International Commission for the Taxonomy of Fungi

Chair: Keith Seifert

0800-1000 **Hall C**

Symposium 42: Genomes and Fitness

Chairs: Gareth W. Griffith (UK) / Simon V. Avery (UK)

The indeterminate growth patterns and diverse reproductive strategies of filamentous fungi make assessment of fitness less straightforward than for some other groups such as animals, though for yeasts fitness is generally equated with rate of cell division . Genomic information now available for many of the best-studied fungi now raises the possibility that the effects of individual genes on organismal fitness can be explored in a way not possible for less tractable experimental higher organisms.

0800-0830 IS3 - 0853

Functional genomics of pathogenicity in Magnaporthe grisea

Nicholas J. Talbot (UK)

0830-0900 IS1 - 0774

Competition experiments using tagged heterozygous deletant Saccharomyces cerevisiae strains in a grape juice environment

Gareth W. Griffith (UK)

0900-0930 IS2 - 0739

Functions determining yeast fitness during stress, derived from genome-wide haploinsufficiency tests

Simon V. Avery (UK)

0930-0945 PS1 - 0657

Fitness effects of interspecific gene transfer in Ophiostoma

Clive Brasier (UK)

0945-1000 PS2 - 0792

Exploring the mobility of DNA transposons in the Dutch elm disease fungi

Guillaume F Bouvet (Canada)

0800-1000 **Meeting Room 3-5**

Proffered Session 1: Phylogeny I

Chair: Heide-Marie Daniel (Belgium)

Molecular phylogenies will be discussed in the context of morphological and other data, including the ecology and geographical distribution of taxa in this session. A common topic of the presentations will be the utilisation of phylogenies to evaluate data for their suitability to build natural classifications and to assess fungal diversity. A strong emphasis is laid on basidiomycetous taxa.

0800-0820 PS1 - 0756

Demystifying Dothideomycetes - combining ultrastructure and molecular tools to study phylogenetic relationships of fungi

Uwe K Simon (Germany)

0820-0840 PS2 - 0453

The molecular phylogeny of the genus entoloma

Delia Co (The Netherlands)

0840-0900 PS3 - 0429

Rust of Salix species in North America caused by Melampsora epitea s. lat.

JA Smith (USA)

0900-0920 PS4 - 0173

Partial harmony: agreement between morphological and molecular data for the sequestrate cortinarioid fungi

Anthony A Francis (Australia)

0920-0940 PS5 - 0512

Phylogeny of Armillaria species based on combined DNA sequence and phenotypic data

Martin PA Coetzee (South Africa)

0940-1000 PS6 - 0379

A polythetic approach to the taxonomy and phylogeny of the Hypoxyloideae

Marc Stadler (Germany)

0800-1000 **Hall D**

Proffered Session 2: Medical Mycology

Chair: Sharon Chen (Australia)

This session welcomes attendees to a series of presentations on fungal pathogens in humans ranging from Aspergillus in high-risk patients to emerging infections caused by novel black yeasts and dermatophytes. The epidemiologic, clinical and molecular taxonomic aspects are addressed.

0800-0825 PS1 - 0054

Evaluation of the Effects of Incubation Temperature and pH on the Antifungal susceptibility Test Against Candida albicans PTCC 5027 Strain

Hossein Zarrinfar (Iran)

0825-0850 PS2 - 0068

Aspergillosis in High Risk Patients

Basiri Jahromi (Iran)

0850-0910 PS3 - 0266

Isolation of Ochroconis gallopava from Hot Springs in Japan and its pathogenicity.

Ayako Sano (Japan)

0910-0935 PS4 - 0593

Genetic diversity and detection of the neurotropic black yeast Exophiala dermatitidis

Montarop Sudhadham (The Netherlands)

0935-1000 PS5 - 0069

Outbreak of Tinea Corporis Gladiatorum in Tehran

Basiri Jahromi (Iran)

1015-1115 - 0762

Plenary 4: Mating in Fungi

Regine Kahmann (Germany)

1145-1345 **Meeting Room 1&2**

Symposium 36: Straminopiles: Why and How?

Chairs: Daiske Honda (Japan) / Gordon Beakes (UK)

The biflagellate 'stramenopile' fungi belong to the same lineage of organisms as the brown pigmented algae. The two main fungal lineages are the Thraustochytrids and Oomycetes. This symposium will explore some of recent insights into the biology and evolutionary origins of these organisms. As well as being significant pathogens in the marine environment, they have the ability to produce sought after unsaturated fatty acids and play key roles in litter colonisation and recycling in many marine and freshwater ecosystems. This session will explore some of these aspects in both the main stramenopile lineages.

1145-1150

Introductory overview of stramenopile fungi

Daiske Honda (Japan)

1150-1215 IS1 - 0772

Evolution and phylogeny of the Labyrinthulomycetes inferred from protein-coding genes

Clement Tsui (Australia)

1215-1225 IS2 - 0481

Taxonomical reinvestigation of the genus Schizochytrium (Thraustochytriaceae, Labyrinthulomycetes)

Rinka Yokoyama (Japan)

1225-1255 IS3 - 0473

The Viral impact on thraustochytrids

Yoshitake Takao (Japan)

1255-1315 IS4 - 0284

The diversity of oomycete pathogens of nematodes and its implications to our understanding of oomycete phylogeny

Gordon Beakes (UK)

1315-1330 PS1 - 0280

Molecular phylogeny and comparative ultrastructural morphology of marine oomycete endoparasites

Satoshi Sekimoto (Japan)

1330-1345 PS2 - 0671

Study Of Mechanism Of Zoospore Release In Stramenopiles Through Videoclips

Anagha Kurne (India)

1145-1345 Meeting Room 3-5

Symposium 37: Advanced Cellular Imaging and Micromanipulation

Chair: Nick Read (UK) / Rosa Mouriño-Pérez (Mexico)

In recent years, there has been a renaissance in the use of imaging and micromanipulation techniques in cell biology. Major innovations and developments in microscope technologies (e.g. confocal microsocopy, 2-photon microscopy), live-cell imaging, fluorescent probes (e.g. GFP), electron microscopy (e.g. field emission EM, electron tomography), and cellular micromanipulation techniques (e.g. laser tweezers, laser microdissection) are having a big impact in fungal biology. This Symposium aims to highlight some of the most recent examples of these exciting new developments in cellular imaging and micromanipulation.

1145-1205 IS1 - 0833

In Vivo Imaging of the Dynamics of the Microtubular Cytoskeleton of Neurospora crassa Wild Type, ropy-1, ropy-3 and nkin

Rosa Mouriño-Pérez (Mexico)

1205-1225 IS2 - 0486

Visualization of the endocytic pathway and endosomal structures in the filamentous fungus Aspergillus oryzae

Yujiro Higuchi (Japan)

1225-1245 IS3 - 0727

NETWORK structure and dynamics of fungal mycelia

Dan Bebber (UK)

1245-1305 PS1 -

Advanced microscopic imaging coupled with X-ray absorption spectroscopy to characterise fungal metal and mineral transformations

Geoff Gadd (UK)

1305-1320 PS2 -

Optical tweezer micromanipulation of filamentous fungi

Nick Read (UK)

1145-1345 Hall C

Symposium 38: Fungal Pigments and Virulence

Chairs: Josh Nosanchuk (USA) / Beatriz L. Gomez (UK)

Melanins pigments are enigmatic compounds that are produced by organisms in all biological kingdoms, including a wide variety of pathogenic bacteria, fungi, and helminthes. Melanin synthesis has been associated with virulence for a variety of pathogenic microbes and this phenomenon has been extensively examined in fungal pathogens. Dr. Beatriz Gomez- Giraldo will describe the identification of melanin in *Candida albicans* and other pathogenic fungi. The third major talk will be by Dr. Josh Nosanchuk who will discuss the clinical significance of fungal melanization. The purpose of this symposium is to provide broad insights into the role of melanins in fungal pathogenesis.

1145-1215 IS1 - 1006

Clinical impact of fungal melanization

Josh Nosanchuk (USA)

1215-1245 IS2 - 1009

The darker side of Candida albicans and Paracoccidioides brasiliensis

Beatriz L. Gomez (UK)

1245-1315 PS1 - 0803

Production and utilization of fungal pigment in textile dyeing

Karuppan Perumal (India)

1315-1345 PS2 - 0340

Peroxisomal acetyl-CoA is essential for appressorial melanization, and virulence in Magnaporthe.

Naweed Naqvi (Singapore)

1145-1345 Halls A&B

Symposium 39: Biosynthetic Gene Clusters for Fungal Secondary Metabolites

Chairs: Nancy Keller (USA) / Marc Stadler (Germany)

Secondary metabolites (extrolites) are of utmost importance in higher fungi. They constitute essential features of high ecological, pathological, and taxonomic significance and exert great detrimental as well as beneficial influence on human civilization. Only recently has it become possible to study their biogenesis at the molecular level, due to the availability of molecular methods and the templates provided by genomic approaches. While most of the research has so far been done on industrial & agriculturally important fungi (in particular, pharmaceutical and mycotoxin - producing ascomycetes), it now appears feasible to study large taxonomic groups in an attempt to evaluate evolutionary aspects of secondary metabolite biosynthesis. In the symposium, different chemical types of metabolites (e.g. polyketides, alkaloids) and different producer organisms will be presented to provide an overview on our current understanding of fungal secondary metabolitsm and future perspectives in the study of these compounds.

1145-1215 IS1 – 0725

The sirodesmin biosynthetic gene cluster of the plant pathogen, Leptosphaeria maculans

Barbara Howlett (Australia)

1215-1245 IS2 - 0240

Terrequinone biosynthesis in Aspergillus nidulans

Dirk Hoffmeister (Germany)

1245-1315 IS3 - 0401

Fumonisin mycotoxin biosynthesis, genetics and genomics in Fusarium verticillioides

Robert Butchko (USA)

1315-1330 PS1 – 0436

Evolution of polyketide synthase genes in lichenized Ascomycetes

Thorsten Lumbsch (USA)

1330-1345

Summary, Future Views and Discussion

Nancy Keller

1145-1345

Symposium 40: Biosecurity

Chairs: Geoff Ridley (New Zealand) / Ceri Pearce (Australia)

Biosecurity is a major issue around the world but particularly for island nations such as Australia and New Zealand. In New Zealand Biosecurity has been defined as "the exclusion, eradication or effective management of risks posed by pests and diseases to the economy, environment and human health". This symposium will cover one countries experience in implementing Biosecurity system. It will also look at the tools available to assist in the identification of potential invasive fungi, and finally how an invasive, disease causing fungus was eradicated.

1145-1215 IS1 - 0917

The importance of mycology in Biosecurity: The New Zealand experience

Mike Ormsby (New Zealand)

1215-1245 IS2 - 0796

Biosecurity: latest developments in systems and tools for fungal diagnostics

Mary Palm (USA)

1245-1315 IS3 - 0961

A major exotic disease outbreak, emergency response and eradication: banana black Sigatoka, Tully, Australia, 2001

Peter Whittle (Australia)

1315-1330 PS1 - 0484

The utility and limitations of positive and negative controls for PCR detection of quarantine pathogens

Morag Glen (Australia)

1330-1345 PS2 - 0634

DISSEMINATION of aerial and soilborne Phytophthoras by human vectors

Joan Webber (UK)

1430-1530

Poster Session 4: Cell Biology and Physiology ***Hall 2***

Poster Session 8: Population Genetics ***Mezzanine Level***

1530-1730 **Meeting Room 1&2**

Symposium 41: Evolution, Ecology and Systematics of Endophytic Fungi - Horizontally Transmitted Endophytes

Chairs: A. Elizabeth Arnold (USA) / Gerard J. M. Verkley (The Netherlands)

Fungal endophytes - fungi inhabiting apparently healthy plant tissues - are known from all plants, and represent a major component of fungal diversity at a global scale. Most studies of fungal endophyte evolution and ecology have focused on the clavicipitaceous endophytes of grasses, whose vertical transmission, systemic growth, low within-host diversity, and benefits to hosts have made them a model system for the study of symbiosis. However, the horizontally transmitted endophytes that occur in all major lineages of plants — inhabiting tissues such as roots, xylem, bark, foliage, and reproductive structures — are ubiquitous in terrestrial communities and are yet largely unknown in terms of their phylogenetic affinities, host distributions, and ecological roles. In this symposium, we seek to bring together researchers whose focus on the systematics, ecology, and evolution of horizontally transmitted endophytes will synthesize our current understanding of these poorly known symbioses, and define major questions for future research.

1530-1600 IS1 - 0605

Host Specificity among endophytes in transient plant communities

George Carroll (USA)

1600-1630 IS2 - 0691

Foliar endophytes versus leaf litter saprobes: annual cycle of an ascomycete community associated with oak leaves

Gerard J. M. Verkley (The Netherlands)

1630-1700 IS3 - 0813

Endophytes: Lifestyle and Phylogenetic Diversity

Rajesh Jeewon (China)

1700-1715 PS1 – 0411

Endophytic fungi in non-mycorrhizal oak roots

Erhard Halmschlager (Austria)

1715-1730 PS2 - 0639

Metabolic and taxonomic approaches to investigating the effects of plant function on communities of root and nodule-associated fungi

Samuel Skinner (Canada)

1530-1730 Meeting Room 3-5

Symposium 56: Phylogeography

Chairs: Gregory Mueller (USA) / H. Thorsten Lumbsch (USA)

The study of processes controlling geographic distributions of lineages using molecular tools is a relatively new and emerging field in mycology. While it was previously generally believed that fungi have wide distribution patterns and have largely unstructured populations, recent studies have shown that this not the case. Phylogeographical approaches offer a powerful tool to understand the current distributions of fungi and their historical development. In this symposium phylogeographical studies on a wide variety of fungal organisms, including smuts, higher basidiomycetes, and ascomycetes, including lichen-forming fungi, will be discussed. The symposium includes examples of parasitic, symbiotic and saprophytic systems. Various molecular markers, such as microsatellites or DNA sequence data of variable gene regions are employed in the different studies.

1530-1600 IS1 - 0370

Phylogeography of Serpula lacrymans reveals global migration events and multiple transitions to an indoor lifestyle

Havard Kauserud (Norway)

1600-1630 IS2 - 0921

Biogeography of the Hysterangiales

Kentaro Hosaka (USA)

1630-1700 IS3 - 0613

Hitchhiking through the botanic realm: Ustilaginales in time and space

Dominik Bergerow (Germany)

1700-1715 PS1 - 0304

A phylogenetic and phylogeographic approach to delimit Antarctic and bipolar species of the genus Usnea, Neuropogon

Nora Wirtz (Germany)

1715-1730 PS2 - 0437

Migration in space and time for 14 worldwide populations of Mycosphaerella graminicola

Soren Banke (Switzerland)

1530-1730 Hall D

Symposium 43: Biocontrol

Chairs: Augusto Schrank (Brasil) / Naresh Magan (UK)

This Symposium will focus on same of the main areas of the biocontrol using filamentous fungi and yeasts. The use of living organisms to control pests and diseases has attracted increasing interest as a reliable alternative to chemical control. In particular, the use of fungi in a commercial scale has proven to be effective and economically feasible in different countries. Filamentous fungi and yeasts have been proposed as biological control agents (BCAs) for a variety of organisms as insects, ticks, phytopathogenic fungi, mycotoxin producers, nematodes. The main difficulty to the large-scale use of fungi as BCAs is the longer time required for effective pest control in comparison to that of chemicals. Much of the current research efforts is applied to improve the knowledge on the host infection mechanisms and to develop optimized formulations. In addition, many groups are involved in discovery of new isolates, a pivotal step towards a more general and efficient application of the biocontrol.

1530-1550 IS1 - 0822

Production and formulation of antagonists for improved competitiveness and biocontrol"

Naresh Magan (UK)

1550-1610 IS2 - 0842

Screening of biocontrol agents of fungal leaf diseases

Jürgen Köhl (The Netherlands)

1610-1630 IS3 - 0987

Strategies to improve Metarhizium control of arthropod pests

Tariq Butt (UK)

1630-1650 PS1 - 0184

Trichoderma spp. and Gliocladium catenulatum associated with Helicobasidium mompa and Rosellinia necatrix

Naoyuki Matsumoto (Japan)

1650-1710 PS2 - 0718

Effect of antagonistic fruit-borne yeasts on pathogenic and saprophytic fungi

Matthias Sipiczki (Hungary)

1710-1730 PS3 - 0154

Nematicidal Metabolites From Fungi

Guohong Li (China)

1530-1730 **Hall C**

Symposium 44: Industrial Mycology

Chairs: Cees van den Hondel (The Netherlands) / Lene Lange (Denmark)

In future, knowledge-based bioeconomy will play a significant role globally. The basis of bioeconomy is the development of biological solutions (both processes and products) to important problems, hereby providing sustainable alternatives to fossil energy and the wealth of petrochemistry products now dominating. And for this to come thru fungal research and development is absolutely central: Fungi for biological production by fermentation. Fungal products in new types of biorefineries. Fungi as pool for discoveries of new active molecules (proteins, peptides, and metabolites). And fungal enzymes for modification of natural substrates; and fungi and fungal products for upgrading Waste to Value. The IMC8 session on industrial mycology will be presenting selected highlights, illustrating the methodologies, the potentials and the diversity of fungi for industrial use.

1530-1600 IS1 - 0026

Diversity of Xylanase and Plant Cell Wall Esterases in Thermophilic and Thermotolerant Fungi

Bhupinder Chadha (India)

1600-1630 IS2 - 1007

New Fungal discoveries –of industrial relevance for biofuel and biopharma

Lene Lange (Denmark)

1630-1700 IS3

Fungal Cell wall biosynthesis and discovery of antifungals

Cees van den Hondel (The Netherlands)

1530-1730 **Halls A&B**

Symposium 45: Worldwide Movement of Fungal Forest Pathogens

Chairs: Brenda Wingfield (South Africa) / Matteo Garbelotto (USA)

The movement of fungal pathogens globally is a source of concern for both plantation as well as native forest systems. The threat of forest pathogens is a very complex one, in some circumstances these pathogens are apparently native and have been able to adapt and cause disease on an introduced host. In other situations fungal pathogens have been introduced into regions where they did not previously occur. Movement of such pathogens has been severely exacerbated with the increase in foreign trade internationally. In this symposium we highlight the current status of a number of internationally important forest pathogens and have invited talks from speakers in both the Northern and Southern hemispheres to give a truly global perspective.

1530-1555 IS1 - 0569

Cryphonectria canker of Eucalyptus: A little-known disease caused by an assemblage of fungi of extreme quarantine relevance

Brenda Wingfield (South Africa)

1555-1620 IS2 – 0585

Global Distribution and Evolution Of The Pine Pitch Canker Fungus, Fusarium circinatum

Emma Steenkamp (South Africa)

1620-1645 IS3 – 0638

Microsatellite analysis documents worldwide and regional spread routes of the sudden oak death pathogen

Matteo Garbelotto (USA)

1645-1710 IS4 – 0270

Invasion of an exotic root pathogen of forest trees: the case of Heterobasidion annosum

Paolo Gonthier (Italy)

1710-1720 PS1 – 0320

Movement of the devastating Eucalytpus leaf and shoot pathogen Phaeophleospora destructans, throughout Asia

Treena Burgess (Australia)

1720-1730 PS2 – 0452

Phaeocryptopus gaeumannii and Swiss needle cast disease in New Zealand

Jeffrey Stone (USA)

1730-1830

Poster Session

1900-2330 **The Tanks**

Congress Dinner

0800-1000
SYMPOSIUM 42 - *Genomes and Fitness*

S42IS1 - 0774
Competition of heterozygous deletant *Saccharomyces cerevisiae* strains in a grape juice environment
G.W. Griffith1, E.J.M Cross1, H.M. Davey1, D. Delneri 2, D.C. Hoyle2, D.B. Kell 2, S.G. Oliver 2
1University of Wales Aberystwyth, Wales, United Kingdom, 2 University of Manchester, Manchester, United Kingdom

The existence of a complete set of 6000 single-gene deletant strains of the yeast *Saccharomyces cerevisiae* represents an important tool for qualitative and quantitative analysis of gene function. The presence of unique barcodes and a selective marker in each deletion cassette allows parallel analysis of strains by microarray *hybridisation*. Strain abundance can thus be quantified as a means of assessing the contribution of individual genes to fitness under a range of environmental conditions. Using a pool of diploid heterozygous deletants, it was possible to identify strains which were either haploinsufficient or haploproficient (i.e. decreased or increased fitness respectively when one rather than both gene copies were present) under different fermentation conditions.
'Competition' experiments were conducted for up to 30 generations, in either continuous or semi-batch fermentor systems, using red /white juices and in the presence and absence of competitor organisms. Changes in fitness identified in grape juice were compared to responses in synthetic nutrient limited media. Whilst fruit juices are generally N-limited, there was incomplete overlap between the strains showing altered fitness in juice vs. N-limited media. Comparison of continuous and semi batch fermentations suggested that growth rate in exponential phase was not the only determinant of fitness.

S42IS2 - 0739
Functions determining yeast fitness during stress, derived from genome wide haploinsufficiency tests
S.V. Avery1, E. Lodwig1, S.L. Holland1, T. Sideri1, I. Clarke 2, K. Gkargkas2, D.C. Hoyle 2, D. Delneri 2, S.G. Oliver 2
1University of Nottingham, Nottingham, United Kingdom, 2 University of Manchester, Manchester, United Kingdom

We have performed microarray-based competitive growth assays of >6,000 heterozygous *S. cerevisiae* deletion strains in the presence of different toxic metals and pro-oxidants. The heterozygous collection encompasses mutants in essential gene functions which, combined with the sensitivity of the assay system, should help to give new insight to the mechanism(s) of cellular metal or oxidant toxicity. Strains were grown together for >20 generations in C-limited continuous culture and samples taken at intervals to determine relative strain growth rates, according to the abundances of amplified strain-specific oligonucleotide tags. Media were supplemented with either Cu(NO3)2, Cd(NO3)2, CrO3, H2O2 or diamide, supplied at concentrations that extended doubling time by ~10%. Strains were identified that gave a significant haplo-insufficient or -proficient phenotype in the various treatments. The corresponding genes of interest were distributed among a range of functional groupings, though certain functions were significantly enriched in our data. For example, protein synthesis and protein degradation functions were found to be important determinants of Cr resistance. We have confirmed phenotypes for individual strains and are using independent approaches to test new hypotheses developing from this study.

S42IS3 - 0853
Functional genomics of pathogenicity in *Magnaporthe grisea*
N.J. Talbot, T.A. Richards, D.M. Soanes, M.J. Gilbert, R.A. Wilson, G.K. Bhambra, Z.Y. Wang, Z. Caracuel-Rios
University of Exeter, Devon, United Kingdom

The rice blast fungus *Magnaporthe grisea* causes one of the most serious diseases of cultivated rice. The availability of a full genome sequence for *M. grisea* (Dean et al., 2005) has allowed the first opportunity to define the gene inventory associated with a fungal phytopathogen. *M. grisea* has a more complex secreted proteome than closely related non-pathogenic fungi and also contains a high number of putative G-protein coupled receptor-encoding genes. This indicates that the fungus possesses an enhanced capacity to respond to distinct environmental signals and the ability to secrete large numbers of distinct proteins during pathogenesis. Consistent with this idea, we recently identified a mutant of *M. grisea* that is impaired in polarised exocytosis (Gilbert et al., 2006). This mutant, *?Mgapt2*, is non-pathogenic and also fails to induce a hypersensitive reaction in an incompatible response, suggesting that it is unable to secrete fungal proteins during plant infection that may act as pathogenicity determinants or cultivar-specific elicitors of plant defence responses (avirulence gene products). MgApt2 is a Golgi-associated P-type ATPase that belongs to the aminophospholipid translocase family. We are using a multi-disciplinary approach, involving high throughput gene functional analysis, proteomics, cell biology and analytical biochemistry, to investigate the biology of plant infection by *M. grisea* and to exploit the genomic resources that are now available for its study. Comparative genomic analysis of *M. grisea* with phytopathogenic and free-living fungal species is central to this process and allows us to explore the evolutionary relatedness of the gene inventories of *M. grisea* with other pathogenic species.
References
Dean, R.A. et al. (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* 434 :980-86
Gilbert, M.J., Thornton, C.R., Wakley, G.E., Talbot, N.J. (2006) A P-type ATPase required for rice blast disease and induction of host defence *Nature* 440: 535-539

S42PS1 - 0657

Fitness effects of interspecific gene transfer in *Ophiostoma*

C. M. Brasier, M. Paoletti, K. W. Buck, A. Et-Touil, L. Bernier, S. A. Kirk
1 Forest Research Agency, Farnham, Surrey, United Kingdom, 2 Imperial College, London, United Kingdom, 3 Imperial College, London, United Kingdom, 4 Universite Laval, Sainte Foy,Quebec, Canada, 5Universite Laval, Sainte Foy,Quebec, Canada, 6Forest Research Agency, Farnham, Surrey, United Kingdom

Interspecific gene transfer offers the potential for rapid evolution of fungi. This potential may be highest under conditions of episodic selection. The anciently divergent elm pathogens *Ophiostoma ulmi, O. novo-ulmi* and *O. himal-ulmi* probably evolved in separate bio-geographic areas of Asia. Laboratory crosses are possible between them; and a degree of natural hybridisation is now occurring through recent intermixing of these species by man. F1 progeny of experimental crosses between the three species exhibit strongly negatively skewed distributions for critical fitness characters such as pathogenicity, growth rate and fecundity, indicating incongruity between the parental genomes. AFLP data indicate that genomes of hybrid progeny are not skewed towards the parental types.
Where *O. ulmi* and *O. novo-ulmi* are intermixing in nature, *O. novo- ulmi* is rapidly replacing *O. ulmi*. Rare hybrids also occur. Some surviving *O. novo-ulmi* isolates carry introgressed *O. ulmi* DNA, and their pathogenicity (fitness) is negatively correlated with the amount of *O. ulmi* DNA present (AFLP data). Transfer of major genes from *O. ulmi* to *O. novo-ulmi* has also occurred, probably as a result of back-crosses between the introgressants and *O. novo-ulmi*. Genes transferred include *O. ulmi* pathogenicity, cerato-ulmin 'toxin', mating type and vegetative compatibility genes. Combined surveys and laboratory studies show (a) a negative effect on fitness of the acquisition of *O. ulmi* toxin or pathogenicity genes by *O. novo-ulmi* and subsequent loss of these genes from *O. novo-ulmi* populations via selection; (b) a strong fitness advantage conferred by acquisition of *O. ulmi* mating type and vegetative compatibility genes and the subsequent widespread fixation of these genes in *O. novo-ulmi* populations.
In broad terms, *O. novo-ulmi* appears to accept 'useful' *O. ulmi* DNA that allows it to survive and to discard 'unwanted' *O. ulmi* DNA that confers reduced fitness. This phenomenon has considerable implications for the adaptation of invasive fungi to new or disturbed environments. It is probably the first demonstration of interspecific transfer of major regulatory genes in a eukaryote.

S42PS2 - 0792

Exploring the mobility of DNA transposons in the Dutch elm disease fungi

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We have cloned and sequenced the first DNA transposons in the Dutch elm disease (DED) pathogens *Ophiostoma ulmi* and *O. novo-ulmi* in order to unravel their genomic implications in the evolution of these ascomycete fungi. Two closely related transposons, named Ophi1 and Ophi2, were found to contain potential spots for positive selection. One of this spots corresponded to the first alpha helix of the Helix-Turn-Helix domain (HTH-psq), implicated in TIR specificity of the transposase and mobility of the transposon. Mobility of fungal transposons can be investigated from two perspectives : mobility inside the host genome or between different host strains or species. We thus conducted experiments in which different stresses, such as thermal shocks, UV irradiations and inoculations to elm tissue, were applied to DED strains carrying transposons. Southern analyses were carried out to assess whether the transposons had moved or not within the host genome. We also analyzed a natural *O. ulmi x O. novo-ulmi* hybrid and found out that it could serve as genetic bridge to allow DNA transposons to jump between species.

PS1PS1 - 0756

Demystifying Dothideomycetes – combining ultrastructure and molecular tools to study phylogenetic relationships of fungi

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The Dothideomycetes (Ascomycota) is a major group of fungi that includes numerous phytopathogenous species and some human and animal pathogens with an enormous variety of life-styles. Despite their economic and scientific importance many taxonomic questions within this group remain unsolved at almost all taxonomic levels from circumscription of orders to species delimitation. With the extraordinary interaction of *Cymadothea trifolii*, an extracellular obligate biotrophic leaf pathogen, and its *Trifolium* host plants as a starting point we examine if the combination of ultrastructural and molecular data can deepen our understanding of phylogenetic relationships within the Dothideomycetes.

First, observations of the cellular interaction of high-pressure frozen and low-temperature embedded samples of *C. trifolii* in *Trifolium* leaves are presented including results from immunocytochemical experiments with respect to differential host cell wall degradation. These findings are compared with ultrastructural observations from other plant-pathogen interactions occurring in the Dothideomycetes. Finally, new nuSSU rDNA sequence data for *C. trifolii* are integrated into phylogenetic analyses based upon a representative sampling of 120 Dothideomycetes.

C. trifolii obtains nutrients from its *Trifolium* hosts by means of a complex interaction structure. Remaining outside host cells, the pathogen forms an intricate interaction apparatus in its own cells. Opposite this structure, the host plasmalemma is triggered to produce a so-called bubble. In an extremely small area (ca. 400 nm wide) between interaction apparatus and host bubble, the host cell wall is partially degraded: The pectin matrix is dissolved by fungal enzymes, while cellulose and xyloglucan remain intact. Electron microscopic studies of other plant-pathogenic Dothideomycetes have revealed a large variety of cellular interactions, but no structures resembling the one produced by *C. trifolii*. NuSSU rDNA phylogenetic analyses show that *C. trifolii* clusters with the *Mycosphaerella punctiformis* group.

The cellular interaction between *C. trifolii* and its *Trifolium* hosts is at present unique among the ascomycetes. Furthermore, it is so far the only extracellular biotrophic fungus for which a highly localized differential host cell wall degradation could be shown. Other fungi placed in the Dothideomycetes exhibit intracellular hyphae or haustoria-like structures. Subcuticulous or intercellular pathogens with no obvious interaction structures are also present. Previous studies about the Exobasidiomycetes (Basidiomycota) have demonstrated that cellular interactions in plant pathogenic fungi are a valuable tool for the reconstruction of phylogenetic relationships of such fungi, which has been proven by molecular data. It is our objective to achieve the same for the Dothideomycetes. This should be a point worthwhile to discuss with all scientists interested in the systematics of fungi.

PS1PS2 - 0453

The molecular phylogeny of the genus *entoloma*

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Entoloma is a large genus characterised by pinkish, variously angular spores and large diversity in fruitbody characteristics. Phylogenetic relationships in the genus *Entoloma* were investigated using sequence data from partial sequences of three genes: RNA polymerase II second largest subunit (nRPB2), 28S ribosomal RNA (nLSU) and mitochondrial small subunit ribosomal RNA genes(mtSSU). Sequenced were representatives of sections according to Noordeloos' classification of European *Entoloma*, species with ambiguous affinities, as well as new, yet unclassified species. Results significantly support that nearly all *Entoloma* subgenera as currently delimited are not monophyletic, with the possible exception of *Pouzarella*. Subgenus *Entoloma* forms a basal grade to the rest the genus except for section *Pseudonolanea*, some of which proves to be truly part of subgenus *Nolanea.* Members of subgenus *Inocephalus* are separated into six distinct clades, reflecting the heterogeneity allowed by the circumscription of this subgenus. Subgenus *Alboleptonia* is separated into four clades, indicating that the character of pale fruiting bodies has been over-emphasised. Despite the polyphyly of *Entoloma* subgenera, some clades still recognisably correspond to subgenera *Nolanea*, *Claudopus*, as well as sections of the subgenera *Leptonia, Entoloma* and *Inocephalus*. This study indicates the need for the re-evaluation of the infrageneric taxonomy of *Entoloma* and the characters used to construct it.

PS1PS3 - 0429

Rust of Salix species in North America caused by *Melampsora epitea* s. lat.

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Melampsora epitea s. lat. is the causal agent of rust disease of willows (*Salix* spp.) throughout North America. Despite abundant evidence for host-level speciation within the *M. epitea* species complex, the current taxonomic literature maintains *M. epitea* as the collective taxon for all willow rusts in North America. Here, we describe studies on the phylogeography, population genetics and co-evolution of these fungi in North America. Investigations include the characterization and sequencing of ITS-rDNA, morphological analyses and AFLP profiles of rust isolates from a broad range of host species, including a comprehensive comparison of Arctic and alpine species in *Salix* section Chamaetia. The results show distinctive host species-delineations and in general, geography is less meaningful in phylogenetic comparisons. Several new putative species are characterized, including a rust causing severe damage and mortality to an endangered species *Salix arizonica*. The results presented indicate that the *Salix-Melampsora* pathosystem in North America is highly diverse and further co-evolutionary studies aimed at comparing host and pathogen phylogenies and host specialization are warranted.

PS1PS4 - 0173

Partial harmony: agreement between morphological and molecular data for the sequestrate cortinarioid fungi

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Analyses of macroscopic and microscopic fruit body morphology and ITS nuc-rDNA sequences are commonly applied techniques for assessing the diversity of cortinarioid sequestrate fungi. Do these methods provide comparable assessments of diversity and relatedness for these fungi? We present the results of combined and separate analyses of morphological and molecular datasets based on examination of 300 herbarium collections of Australian cortinarioid sequestrate fungi. Analyses were, of necessity, based on a low number of morphological characters and variable-quality field descriptions. In general however, groups of species defined on the basis of the morphological dataset coincided with groups determined from the ITS and combined datasets. Certain taxa consistently grouped together in all analyses of all datasets; while others were allied with different fungi when different datasets were considered. The agreement between morphology and ITS data for a given taxon appears dependant on which characters defined that taxon. These observations support the contention that, for these fungi, certain characters, including peridial and spore characteristics as they are commonly defined, are polyphyletic – as has been indicated for agaricoid Cortinarius species. Such polyphyletic characters, while useful diagnostically, may be of limited use phylogenetically or may require more detailed characterisation before the relationships between analogous characters can be confidently traced.

PS1PS5 - 0512

Phylogeny of *Armillaria* species based on combined DNA sequence and phenotypic data

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Species of *Armillaria* cause root rot on a wide variety of trees and plants valuable to the forestry, agricultural and horticultural industries, world-wide. The importance of these fungi as pathogens has justified numerous studies to elucidate their taxonomy and phylogenetic relationships. Past studies have typically been based on single gene analyses and have produced incongruent phylogenies. The aim of this study was to reconstruct the phylogenetic relationships among a global collection of *Armillaria* spp. using phenotypic and DNA sequence data, combined to form a super-matrix. Data were obtained from previous publications or, when not available elsewhere, determined as part of this study. Analyses were based on Bayesian phylogenetic tree search methods. *Marasmius alliaceus* was used as outgroup taxon. The phylogram generated from the analysis, separated isolates into two groups with high posterior probabilities. The one group included all isolates representing species from the Northern hemisphere and the other those from the Southern hemisphere. The only exception to this grouping was for isolates of *A. hinnulea,* which grouped apart from the two clusters. Within the Northern hemisphere collections, *A. mellea* and *A. tabescens* grouped together by virtue of a shared deeper node. High intra- and inter-specific character variation was observed for this species group, suggesting that they are ancestral to remainder of the species from the Northern hemisphere. Species in the Southern hemisphere group were separated by branches much longer than those observed for the Northern hemisphere taxa. This suggests that their time of divergence from a common ancestor is much older than those species occurring in the Northern hemisphere. Results of this study, therefore, support the view that *Armillaria* spp. from the Southern hemisphere form an ancient group and that they are ancestral to species from the Northern hemisphere.

PS1PS6 - 0379

A polythetic approach to the taxonomy and phylogeny of the Hypoxyloideae

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The *Hypoxyloideae* (*Xylariaceae* with *Nodulisporium*-like anamorphs) have been revealed to be a rather homogenous group by a combination of morphological studies on their teleomorphs and anamorphs, HPLC profiling using diode array and mass spectrometric detection, and analyses of their ribosomal gene sequences [1-4]. The distribution of chemical types of pigments was also found basically in agreement with the current concept based on an independent study using morphological and molecular data (?-actin and ?-tubulin sequences) that recently resulted in the recognition of *Annulohypoxylon* [5]. This work also emphasised the status of *Daldinia* as a monophyletic group that eventually arose from *Hypoxylon*-like ancestors.
Currently, we are focussing on the affinities of some small taxon groups, such as *Pyrenomyxa* and *Phylacia*, whose teleomorphs show deviations from the "*Xylariaceae* prototype" [5], or on tropical problem taxa in the *Hypoxyloideae*. According to unpublished results, some tropical members of *Hypoxylon* with massive stromata appear more closely related to *Daldinia*, and we also established their affinities to certain anamorphic endophytic *Nodulisporium* spp.
A polyphasic approach appears suited best for evaluation of the biological diversity of, e.g., some highly variable species complexes of *Daldinia* and *Hypoxylon* in the tropics, since old type specimens collected in the 18th and 19th century can be related to recently collected ones by comparing their specific secondary metabolite fingerprints; the recently collected ones may subsequently be used for studies in their anamorphic and molecular characteristics. Despite HPLC fingerprinting alone cannot solve all imminent questions in the phylogeny and taxonomy of these fungi, this technique constitutes an important complementary tool to provide additional informative characters. Soon, HPLC profiling of cultures in conjunction with molecular and morphological data may even shed some further light on the phylogeny of the order *Xylariale*s.

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0800-1000

PROFFERED SESSION 2 - *Medical Mycology*

PS2PS1 - 0054

Evaluation of the effects of incubation temperature and pH on the antifungal susceptibility test against *candida albicans* PTCC 5027 Strain

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Candidiasis, as an opportunistic infection, is created by the *Candida* species.Although *Candida albicans* is classified in the body as endogen flora, it plays an important role in creating *Candida* related diseases. *Candida* infections particularly in pregnant women, diabetic mellitus and the patients using multiple antibiotics and contraceptive drugs, demonstrates high resistance against the conventional medication. On the other hand, the recurrent *Candida* infections disintegrates the long-term process of treatment in majority of the patients. At the present research, which is aiming at determining the optimum conditions in the Antifungal susceptibility testing for Fluconazole, Clotrimazole and ketoconazole before the retreatment of the patients. Strain of *Candida albicans* PTCC 5027 (Persian type culture collection) and powder of Fluconazole, Clotrimazole and ketoconazole, were used at two pH 7.2 and 5.5, and at two temperature 35ºC and 27ºC. The Microdilution broth test technique was performed to do this. The RPMI 1640 medium within the 96 well microplates was used to determine the MIC50 , MIC90 and MFC this drugs. The obtained MIC50, MIC90 and MFC at these conditions (T=35ºC and pH=7.2) for Fluconazole were 0. 25 to 1 µg/ml, 1 to 4 µg/ml and 256 to ?1024 respectively, and for Clotrimazole were 0.125 to 1 µg/ml, 1 to 8 µg/ml, 128 to 512 µg/ml respectively,and for ketoconazole were 0. 25 to 1 µg/ml, 1 to 4 µg/ml, 64 to ?512 µg/ml, respectively. The obtained results confirmed that the temperature of 35ºC and pH 7.2 in comparison to the other conditions, produced better treatment outcomes.

PS2PS2 - 0068

Aspergillosis in high risk patients

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Aspergillus is the first cause of infectious death after transplantation and remains a major complication in curses of leukemia treatment. Despite considerable progress in the management of infections remains an important cause of morbidity and mortality, mainly after transplantation.

A case control study of 24 patients with aspergillosis was done to identify significant risk factors for invasive aspergillosis. Diagnosis was confirmed by demonstration of fungi in direct preparation and culture techniques.

The patients were immunocompetent or of anatomic structure of the infected site. Among patients with solid organ transplantation, renal transplant patients, hematologic malignancy and chronic granolomatous disease were at the highest risk of developing invasive aspergillosis (IA). Fever unresponsive to broad-spectrum antibiotics was the earleast and most common in this study.

The major advances in the management of invasine fungal infections (IFI) have come from the understanding of the risk factors for the development of IFI, from the development of new biological markers of IFI and also from well-designed therapeutic trails. However, much remains to be done to decrease the rise of mortality due to IFI in high risk patients. A high degree of awareness and efforts for an early diagnosis may participate to improve the poor prognosis.

PS2PS3 - 0266

Isolation of *Ochroconis gallopava* from Hot Springs in Japan and its pathogenicity.

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Ochroconis gallopava is a species of dematiaceous fungi recognized as a causative agent of emerging fungal zoonosis. More than 30 human cases including 3 cases from Japan have been reported. The pathogen has caused outbreaks in poultry and wild birds, and a few cases in domestic cats. Environmental isolates of *O. gallopava* have also been found under low-pH and thermal conditions, such as in coal waste piles, hot springs, sewage from nuclear power plants, and broiler-house litter.
The present study tried to isolate *O. gallopava* from hot spring environments in Japan and studied their pathogenicity in mice because our country is famous for volcanoes and hot springs.
Twelve samples of hot spring water corrected from the northern to the southwestern areas of Japan were examined. The pHs of hot springs were approximately 5.6, and the temperatures at the collecting moment were 41-42oC. Five hundreds milliliter of the hot spring water was filtrated with a 0.22 micrometer-pore-sized filter. The filter was cultured at 42oC on potato dextrose agar plates for 2 weeks. Brownish colonies were picked up and studied mycologically and molecular biologically. The D1/D2 domain of large subunit ribosomal RNA gene sequences was compared with the GenBank database. *O. gallopava* isolates from the hot spring environments were examined in their virulence by intravenous injection into mice (5 x 105 conidia /10 g body weight) treated with or without corticosteroid. The behavioral changes, mortality, recovery rate of fungal cells from six organs (brain heart, lung, liver, spleen and kidney), and histopathological observations were recorded up to 28 days after inoculation.
Four isolates of *O. gallopava* were obtained from 2 hot springs located at Kanto area (middle part of Japan). Colonies of the 4 isolates were floccose, and dark olive green on the surface and dark brown in the reverse. The isolates uniformly showed an excellent growth at 42oC and could grow up to 48oC. All the isolates abundantly produced two-celled clavate conidia attached to denticles on conidiophores. Some mice showed rotating movement and sedation after 4 days inoculation irrespective of the isolates. The mortalities in mice were 20 to 100% unrelated to the treatment of corticosteroid. The target organs were the brain, kidney and liver which showed mycelial growth with or without cellular reaction.
This study demonstrated the first isolations of *O. gallopava* from the environments in Japan. Japanese people like hot springs for treatments of chronic diseases, however, not only such patients but also healthy subjects should be aware of the fungus because it caused fatal infections in non-corticosteroid treated mice. The species may cause infections in poultry and wild birds similar to highly pathogenic avian flu and SARS in Japan.

PS2PS4 - 0593

Genetic diversity and detection of the neurotropic black yeast *Exophiala dermatitidis*.

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The black yeast *Exophiala dermatitidis* is an uncommon etiologic agent of fatal infections of the central nervous system in otherwise healthy, mainly adolescent patients in East Asia. The route of infection is still a mystery. The steam bath apparently provides a novel environmental opportunity for this fungus, but it is uncommonly distributed by air or steam and thus inhalative infection from the steam bath is unlikely. Rapid screening of a diversity of environments is therefore essential. Two preponderant ITS rDNA genotypes are known. It is our aim to enhance detection of the genotypes in natural and human-dominated environments.
Strains were isolated by pre-incubation in Raulin's solution, and subsequently on Erythritol-Chloramphenicol Agar (ECA) at 40°C. Strains were purified with Tween 0.1%. For phenetic identification, assimilation of nitrate was tested. Genotype-specific assays were developed using Amplified Fragment Length Polymorphism (AFLP), by Single-Strand Confirmation Polymorphism (SSCP), by restriction analysis (RFLP) and by applying selective primers.
Growth at 40°C and absence of assimilation of nitrate separate *Exophiala dermatitidis* and *E. phaeomuriformis* from all other black yeasts. An RFLP assay for detection of the preponderant genotypes and a genotype with a 30 bp Indel in ITS1 was developed. Digestion with *Taq* I revealed two different types, by which the genotypes I and II could be recognized easily. A third genotype was recognized by a small band shift. Genotype detection was enhanced by the use of specific primers and SSCP. With AFLP a further subdivision of genotypes was possible.
RFLP combined with simple phenetic data enabled precise recognition of *E. dermatitidis* down to the genotype level. Specific PCR assay enable detection without culturing. In combination the methods will enhance large-scale screening of clinical and environmental samples

PS2PS5 - 0069

Outbreak of Tinea Corporis Gladiatorum in Tehran

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In recent years, skin diseases in wrestling have finally received the attention it deserves. Outbreaks of tinea corporis are often associated with sports such as wrestling that involve extensive bodily contact. Tinea corporis gladiatorum, caused in most by *Triclophyton tonsurans*, infect wrestlers at alarming rates. The management of skin infections in wrestlers and other athletes in sports involving skin-to-skin contact is challenging, from making an accurate diagnosis to determining eligibility for play. To control the outbreak, we conducted an epidemiologic investigation. The purpose of this article is to determine the prevalence of tinea corporis gladiatorum in wrestlers club in Tehran.
A study of dermatophytosis among wrestlers was carried out during the period March 2001 to December 2005 in 612 mycologically proven cases of dermatophytosis in wrestlers in Tehran. The wrestler mycologically examination consisting of direct microscopic observation and culture of pathologic material. Diagnosis was based on the macro- and microscopic characteristics of the colonies.
Trichophyton tonsurans was the predominant dermatophyte,accounting for >90% of all tinea corporis gladiatorum isolates in each of the 5 years analysed.Tinea corporis gladiatorum was found to be more frequent in 10-30 age groups (94.6%).The wrestlers with corporis gladiatorum were mostly from wrestler clubs in south and south-east of Tehran.Transmission of tinea corporis is primarily through skin-to- skin contact.
The rapid identification and treatment of tinea corporis gladiatorum is vital to minimize disruption in team practices and competition, are paramount. . Because infection with dermatophytes can disqualify a wrestler from competing in matches, vigilant surveillance and rapid initiation of therapy can reduce the suspension of a team's practice and competition.

1015-1115 – 0762
PLENARY 4

Mating in fungi

Regina Kahmann
Germany

In my lecture I will give a brief overview of the conserved mating systems present in diverse groups of fungi and describe how these loci determine cell identity and promote outbreeding. Special emphasis will be given to the bipolar and tetrapolar mating systems of the Basidiomycetes where multiple specificities arise through recombination. The developmental pathways which are regulated by the mating type genes will be outlined using the smut fungus *Ustilago maydis* as prime example. In this organism the *b* mating type genes control morphogenesis and pathogenic development. In their active form the b proteins exist as heterodimeric transcription factors that trigger a regulatory cascade comprising about 250 genes. Many of these genes have unknown functions and this percentage is especially high among genes whose products are predicted to be secreted. I will present evidence that some of these novel proteins have essential functions during pathogenesis.

1145-1345
SYMPOSIUM 36 - *Straminopiles: Why and How?*

Introductory overview of stramenopile fungi

Daiske Honda
Japan

S36IS1 - 0772

Evolution and phylogeny of the Labyrinthulomycetes inferred from protein-coding genes

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The *Labyrinthulomycetes* are fungus-like protists including *Thraustochytrids*, *Aplanochytrids* and *Labyrinthulids*. They are common marine heterotrophs and parasites, but with huge significance in biotechnology. Previous phylogenies based on SSU rDNA suggested that most *Labyrinthulomycete* genera were not monophyletic. We are applying phylogenies from genes for elongation factor 1a, b-tubulin, and actin, to re-evaluate the relationships among these genera using parsimony, likelihood and Bayesian analysis. Our results from analyses of protein coding genes from twenty-two taxa were congruent with rDNA phylogenies in showing that *Schizochytrium* and *Thraustochytrium* were not monophyletic. Although morphological characters did not necessarily predict phylogeny, taxa sharing similar biochemical features did appear to be monophyletic. We also generated actin, and b-tubulin sequences from two species of Bicosoecida (heterotrophic stramenopiles) to explore their phylogenetic relationships with the *Labyrinthulomycetes* and other eukaryotes. Results from analysis of amino acid sequence alignments showed that the *Labyrinthulomycetes* formed a strongly supported monophyletic sister group to the *Bicosoecida*, within the stramenopiles. As expected, the stramenopiles clustered with the alveolates in a monophyletic clade.

S36IS2 - 0481

Taxonomical reinvestigation of the genus *Schizochytrium* (Thraustochytriaceae, Labyrinthulomycetes)

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The members of the family Thraustochytriaceae have wide distribution and high abundance in the marine environment, so that it has been suggested that they play the important ecological role as the decomposers. In addition, they are also known to produce large amount of polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA), which are considered as the important industrial resources for microbial PUFA production. However, the molecular phylogenetic works clearly showed that the genera in the Thraustochytriaceae did not form monophyletic groups. It has been strongly suggested that the taxonomical rearrangement might be necessary. In this study, we examined the taxonomy of the genus *Schizochytrium*, based on the morphological observations, comparison of chemotaxonomic features, and molecular phylogenetic analyses. First, the *Schizochytrium* strains were selected from originally established strains of thraustochytrids based on the key character of this genus, that is, the forming cell clusters via successive binary divisions. Constructed phylogenetic tree of 18S rRNA gene sequences of selected strains and reported thraustochytrid organisms strongly suggested that all the examined *Schizochytrium* strains separated into three distinct monophyletic groups. The members of the first group with type species, *S. aggregatum*, possess comparatively high ratio of arachidonic acid in the PUFAs and no accumulation of the xanthophylls in the carotenoid pigments. The second group with *S. limacinum* is characterized by accumulation of canthaxanthin and astaxanthin, and high ratio (ca. 80%) of DHA in the PUFAs. The third group with *S. minutum* showed the different profile, which is distinguished by only the canthaxanthin as the xanthophyll pigment and n-3 DPA in the PUFAs. Especially, n-3 DPA is highly specific in not only the genus *Schizochytrium* but also all the labyrinthulomycete organisms. These agreements of the molecular phylogeny and chemotaxonomic characters strongly suggested that three phylogenetic groups are the natural groups, therefore, we proposed the genus *Schizochytrium* should be separated into three genera.

S36IS3 - 0473

The Viral impact on thraustochytrids

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Thraustochytrids are cosmopolitan stramenopile "fungi". They are distributed in saline lakes, marine, estuarine and deep sea waters. The biovolume of thraustochytrids in coastal waters could reach ~43 % of the bacterial biovolume. Their wide distribution and high abundance are of much ecological interest. In addition, thraustochytrids are known to produce large amount of polyunsaturated fatty acids such as docosahexaenoic acid and docosapentaenoic acid, which are considered important as food resources for higher organisms in marine systems. Because of these distinctive features, the ecological importance of thraustochytrids in the coastal ecosystems has recently been recognized; however, either their natural dynamics or ecological roles *in situ* have scarcely been understood. Viruses and virus-like particles (VLPs) are the most abundant bioactive agents in marine environments; now, viral infection is recognized as one of the important factors in controlling the biomass and clonal composition of bacterial and algal populations. While, there are few reports concerning viruses infecting heterotrophic protists. In this study, we measured the dynamics of viruses by MPN assays using 12 thraustochytrid stains as hosts, established and characterized viral strains isolated from the western coast of Japan to discuss the ecological relationships between thraustochytrids and their infectious viruses.

Based on the characteristics of viral strains isolated through the survey, we revealed they are most likely divided into two groups: one is a small icosahedral single-stranded RNA virus (*Schizochytrium* single-stranded RNA virus: SssRNAV [ø25nm]); the other is a large roundish (but not icosahedral) double-stranded DNA virus (Thraustochytrids DNA virus: ThDNAV [ø140nm]). These two virus groups showed significantly different dynamics through the field survey conducted in Hiroshima Bay, Japan in 2004-2005: SssRNAV showed a temporary increase in abundance following *H. akashiwo* blooms; in contrast, ThDNAV remained at a relatively low concentration showing no drastic changes in abundance through the survey. Considering the dynamics of each virus group should reflect the changes in abundance of its host, there are at least two thraustochytrid groups coexisting in Hiroshima Bay that are ecologically different showing dissimilar fluctuation patterns; one that utilizes on dying and dead algal cells and the other mainly functioning as a decomposer for organic matters of land origin. It may be that the two host groups are dominant in the coastal environments, and each of them is affected by a distinct type of virus; i.e., either SssRNAV or ThDNAV.

S36IS4 - 0284

The diversity of oomycete pathogens of nematodes and its implications to our understanding of oomycete phylogeny.

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Most of the biflagellate holocarpic parasites of nematodes were traditionally classified in the Lagenidiales. However, in his recent revision of oomycete systematics, Dick transferred most to a new order, the Myzocytiopsidales which also encompassed a number of holocarpic marine parasites of crustacea. We have recently described the morphological and ultrastructural development of a number of these holocarpic parasites of nematodes encompassing the genera, Myzocytiopsis, Chlamydomyzium and Haptoglossa. We are also trying to use molecular markers to place these organisms within context of the oomcyete phylogenetic tree, although it has proved a challenge to obtain such data for these obligate parasites.

These apparently similar holocarpic organisms show varied types of zoosporogenesis, with some species showing dictyuchoid and achlyoid aplanospore discharge, whilst others have either intra- and extra sporangial differentiation of zoospores. Combined with significant differences in fine-structure between species, the evidence points to the conclusion that the Myzocytiopsidales cannot be considered a natural assemblage. Families and species within this order will undoubtedly need to be assigned elsewhere. It is also apparent that the systematics of these holocarpic parasites needs to be based on both molecular and ultrastructural characters and that light microscopic morphology alone is insufficient.

It does seem likely that these holocarpic parasites will prove pivotal to our understanding of the evolution of the oomycetes. Mycelial saprotrophic or biotrophic groups within the Saprolegniaceae and Peronosporaceae probably evolved from such holocarpic parasitic ancestors.

S36PS1 - 0280

Molecular Phylogeny and Comparative Ultrastructural Morphology of Marine Oomycete Endoparasites

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The class oomycetes is a member of the Chromistan lineage, which includes labyrinthulids, bicosoecid flagellates and heterokont algae etc. Recent molecular phylogenetic analyses have given us an understanding of how the main orders are related and has revealed a basal cluster consisting almost exclusively of marine endoparasites, which branch both before both Saprolegnian and Peronosporalean clades. These marine endoparasites therefore seem to hold the key to unraveling the mysteries of both the phylogenetic origin and evolutionally development of oomycetes. We will summarize our ongoing comparative studies on the ultrastructural morphology of three of these little studied marine oomycete endoparasites (*Olpidiopsis* spp, *Haliphthoros* sp, and *Eurychasma dicksonii)* as part of our approach to deducing the origins of oomycete fungi. Key phylogenetic features we have observed include the short double gyr transitional helix in the flagellar transitional region of the zoospore of *Haliphthoros* sp., K-body-like structure in the zoospores of *Olpidiopsis* sp., and central vacuolation and centrifugal generation of zoospore initials in *E. dicksonii.* These features are shared with many Saprolegnian oomycetes suggesting a closer phylogenetic affinity to these than with Peronosporalean oomycetes. Recent observations of *E. dicksonii,* which is located at the most basal position of oomycetes, show complex patterns of sporogenesis, including two distinct patterns of zoosporogenesis. Typical oogenesis has not been observed in any of these marine species and it is generally stated that they lack sexual reproduction, but it may be that sexual reproduction could be non orgamous. It is clear that zoosporogenesis and zoospore-release mechanisms are significant features reflecting the phylogeny. The complicated patterns of zoosporogenesis seen in Eurychasma indicates considerable morphological complexity and diversity in these early-branched marine holocarpic endoparasites. Much more morphological and molecular data on other unexplored marine holocarpic species is needed to resolve the mystery of the origins and early evolution of the oomycetes.

S36PS2 - 0671

Study Of Mechanism Of Zoospore Release In Stramenopiles Through Videoclips

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Zoosporic Fungi are those fungi, which develop motile zoospores in different types of zoosporangia and release them in surrounding water with well-organized mechanism. This mechanism remarkably differs in different genera and classified and confirmed accordingly. Therefore, zoospore release has a special significance in the study of Zoosporic Fungi. In the present paper, we have confirmed 5 different genera, *Achlya, Saprolegnia, Dictyuchus, Aphanomyces, Olpidiopsis* of the order Saprolegniales from Stramenopiles through videoclips by observing the development of zoospores and the mechanism of their release through the zoosporangia with Olympus Microscope, CH 20 I and CCD camera attachment from the live aquatic cultures.

1145-1345

SYMPOSIUM 37 - *Advanced cellular imaging and micromanipulation*

S37IS1 - 0833

In Vivo imaging of the dynamics of the microtubular cytoskeleton of neurospo4ra crassa wild type, *ropy-1, ropy-3 and nkin*

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By light and electron microscopy techniques, the organization and behavior of microtubules (MTs) were examined in hyphae of *Neurospora crassa* wild type strain and *ropy-1* (dynein), *ropy-3* (dynactin) and *nkin* (kinesin) mutants. In wild-type *N. crassa*, cytoplasmic MTs were abundant and mainly arranged longitudinally along the hyphal tube. Straight segments were rare; most MTs showed a distinct helical curvature with a long pitch and a tendency to intertwine with one another to form a loosely braided network throughout the cytoplasm. Microbutules in the *nkin* mutant showed a very similar behavior than the wild type, although the hyphal growth rate was slower. In both *ropy-1* and *ropy-3* mutants, there was a marked decrease in the number of MTs throughout the distorted hyphal. This decrease was especially evident in the apical region. In the *ropy* mutants, MTs were generally shorter than in the wild type and showed a greater tendency to form bundles. Overall, the MT cytoskeleton of the *ropy* mutants appeared scant and disorganized. In the 3-D images, the helical character of MTs was evident but pitch and orientation relative to the growing axis fluctuated widely. As hyphae elongated, the MTs moved forward in a helical pattern that was more readily apparent in the mutants because of the fewer number of MTs. FRAP studies were performed to evaluate MT assembly dynamics at the growing tip. Microtubule nucleation was observed at the apical plasma membrane of young hyphal branches. In conclusion, the kinesin, dynein or dynactin deficiencies of the nkin and *ropy* mutants cause a perturbation in MTs organization, as previously described, but also in MT dynamic instability with serious negative consequences on hyphal growth rate.

S37IS2 - 0486

Visualization of the endocytic pathway and endosomal structures in the filamentous fungus *Aspergillus oryzae*

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Endocytosis is an important process for cellular activities. However, in filamentous fungi, the existence of endocytosis has been so far elusive. In this study, we used AoUapC-EGFP, the fusion protein of a putative uric acid-xanthine permease with EGFP (enhanced green fluorescent protein) in the filamentous fungus *Aspergillus oryzae*, to examine whether the endocytic process occurs or not. Upon the addition of ammonium into the medium the fusion protein was internalized from the plasma membrane. The internalization of AoUapC-EGFP was completely blocked by sodium azide, cold, and cytochalasin A treatments, suggesting that the internalization possesses the general features of endocytosis. These results demonstrate the occurrence of endocytosis in filamentous fungi. Moreover, we discovered the endosomal compartments that appeared upon the induction of endocytosis. The endosomal compartments displayed intermittent and bidirectional movement longitudinally along the hyphae, in a microtubule-dependent manner. Effects of the deletion of the motor proteins will be also included in the presentation.

S37IS3 - 0727

Network structyure and dynamics of fungal mycelia

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Many physical phenomena, from road systems to the internet, can be modelled as networks. Network analyses have revealed important properties of, and commonalities among, diverse experimental structures. The fungal mycelium is a transport network that competes in a complex and changing environment. The architecture of the network continuously adapts to local nutritional or environmental cues, damage or predation, through growth, branching, fusion and regression. We investigate whether mycelial network architecture optimizes resource capture, exploration, translocation, or defence.

We use image analysis techniques to digitize the growing mycelia of cord-forming saprotrophic fungi, and to assign transport capacities and physical resiliences to the cords (Fig. 1). These models are then analysed using a variety of network-based statistics. We compare modelled transport capacity and routing with real values derived from scintillation imaging of radiolabelled mycelia, and compare *in silico* attack with responses of real mycelia to experimental attack by fungivorous collembola.

As the mycelium grows, some cords thicken to increase transport capacity, such that the network's effective diameter remains static even as its physical size increases. We term this property a 'physiological small world' effect. Differential cord thickening also increases the resilience of the network to attack, by protecting a central core component at the expense of weaker peripheral cords. The network responds to actual physical damage by increasing redundancy in transport routes.

Our analyses have revealed that the fungal mycelium is a self-organised network that balances transport and defence. Further investigation of variation in mycelial structure among fungal species may reveal trade-offs between properties such as exploration and resilience, and suggest adaptations to different ecological niches. Network theory provides a new and exciting way of understanding fungal biology and ecology.

S37PS1

Advanced microscopic imaging coupled with x-ray absorption spectroscopy to characterise fungal metal and mineral transformations

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In this study, scanning electron microscopy (SEM)-based techniques were used to study metal-mineral transformations by fungi, especially the formation of mycogenic minerals, while X-ray absorption spectroscopy (XAS) was used to determine the speciation of metals within biomass. It was found that fungal-mineral interactions at the microscale level could be successfully studied using different SEM approaches which preserved living fungal microstructures and the microenvironment where the precipitation of mycogenic minerals occurred. Environmental scanning electron microscopy (ESEM) in the wet mode was the best method for observing such interactions in their natural microenvironment; X-ray element mapping demonstrated sequestration and localization of metals. Cryo-SEM allowed the observation of both interior and exterior morphology and appeared to preserve the complex structure of ectomycorrhizal roots better than other microscopic techniques. The amorphous state or poor crystallinity of metal complexes within the biomass and relatively low metal concentrations makes the determination of the metal speciation a challenging problem but this can be overcome by using synchrotron-based element-specific XAS techniques. Here, we exposed fungi and ectomycorrhizas to a variety of copper-, zinc- and lead-containing minerals. XAS revealed that oxygen ligands (phosphate, carboxylate) played a major role in toxic metal coordination within fungal biomass during the accumulation of mobilized toxic metals. The use of state-of-the-art SEM techniques and XAS has provided new information about the role of fungi in metal-mineral transformations and their importance in "geomycological" processes.

S37PS2
Optical tweezer micromanipulation of filamentous fungi
Nick Read
United Kingdom

No abstract available.

1145-1345
SYMPOSIUM 38 - *Fungal Pigments and Virulence*

S38IS1 – 1006
Clinical impact of fungal melanisation
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USA

Host and microbial melanins are high molecular weight pigments that can bind diverse compounds, including antimicrobial drugs. For some microbes melanin appears to contribute to virulence by reducing their susceptibility to host defense mechanisms and by altering host immune responses. Microbial melanization can interfere with the activity of antimicrobial drugs in vitro, and may potentially result in clinical resistance, particularly for certain antifungal drugs. Awareness of the effect of melanin on microbial susceptibility to drugs and host defenses may be an important consideration in the selection and development of antimicrobial therapy.

S38IS2 – 1009
The darker side of *Candida albicans* and *Paracoccidioides brasiliensis*
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Melanins are a ubiquitous class of biological pigments, and melanization in many human fungal pathogens is emerging as an important contributor to virulence. Both conidia and yeast cells of the thermally dimorphic fungal pathogen *Paracoccidioides brasiliensis*, the etiological agent of paracoccidioidomycosis (PCM), produce melanin or melanin-like compounds in vitro and in vivo. Our results demonstrate that *P. brasiliensis* synthesizes and polymerizes melanin during infection and generates antibodies, principally IgG but not IgM, against conidia and yeast melanins, as detected in sera and BALs such as reported previously in other fungi. The murine anti-P. brasiliensis melanin MAbs obtained by our group and the significance of antibody response in vivo will be useful in the study of *P. brasiliensis* melanization, particularly in regard to passive immunization for prolonged survival of infected mice and/or modulation of the immune response against *P. brasiliensis* infection.
Ongoing work has now shown that *C. albicans* yeast cells can produce melanin-like material in vitro and in vivo and they also have the enzymatic machinery required for melanization. Melanin particles were isolated after the digestion of fungal cultures using enzymes, denaturant and hot concentrated acid. The particles were confirmed as melanin by scanning and transmission electron microscopy and electron spin resonance spectroscopy. Novel anti-melanin monoclonal antibodies (Mabs) were then generated against melanin and these were then used to further investigate the role of melanin in tissue infections in each fungal species investigated. Minimum inhibitory and minimum lethal concentrations of antifungal drugs were determined for melanin-containing and melanin-deficient strains, showing that melanin may play a role in resistance to killing by drugs in vitro. We are currently investigating any potential role of melanogenesis in *C. albicans* in the pathogenesis of infection.

S38PS1 - 0803
Production and utilization of fungal pigment in textile dyeing
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Colour is essential part of nature. Recently due to the hazardous nature of synthetic dyes, there is an increasing interest in using micro organisms as colour source. Five color (orange red, red, orange, yellow and brown) producing fungi *Amanita muscaria, Ganoderma lucidum* and *Coriolus versicolor, Boletus edulis (yellow)* and *Armillaria tabescens (orange color)* were collected, identified, optimized for their pigment yield and pure mycelial cultures were established. The fungal pigment was extracted (250 gm / 1000 ml) by suitable solvents (water, ethanol). The pigment subjected to UV- spectral analysis, estimation of protein content and anti-microbial assay. The extracted pigments were applied on cotton yarns using various mordant, which resulted in various shades such as yellow, orange and brown color. The dyed yarns did not alter colour on solar drying and repeated washing in tap water. Among the seven different substrates (saw dust, maize, wheat sorghum, paddy husk and pearl millet), maize grains was the best substrate for spawn development of *Ganoderma lucidum* whereas a combination of sorghum and paddy husk had given a better spawn growth for *Coriolus versicolor*.
Invitro cultivation of *Coriolus versicolor and Ganoderma lucidum were* explored by utilizing sugar cane bagasse, wood shavings, saw dust, paddy husk, banana leaves, dried moringa stems and paddy straw. Among these raw materials, sugarcane bagasse was suitable for production of *Ganoderma lucidum* fruit body within a period of 35 days. Fifteen bags of 1.5 kg each have been prepared using 20 kg of sugarcane bagasse which in turn had a yield of 100 to 200 gm per bag. The bio-efficiency of fruit body production was found to be 20 to 30%. Fruit body was subjected to pigment extraction using hot water and obtained brown pigment powder by lyphilisation process (4%).

S38PS2 - 0340

Peroxisomal acetyl-CoA is essential for appressorial melanization, and virulence in *Magnaporthe*

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The long-chain fatty acids undergo beta-oxidation primarily in the peroxisomes and the resultant acetyl-CoA molecules (and the chain-shortened fatty acids) are exported via the cytosol to the mitochondria for further breakdown and usage. In a forward genetics approach, we identified a loss-of-function mutation in the *Magnaporthe grisea PEROXIN6* locus. Disruption of PEX6 function led to total nonpathogenicity. Further characterization revealed that *Mgpex6-delete* strain lacks functional peroxisomes and is incapable of beta-oxidation of long-chain fatty acids, and that the peroxisomal acetyl-CoA is essential for the host invasion step of the rice-blast disease. The *Mgpex6delete* lacked appressorial melanin and could not elaborate penetration hyphae, and was thus rendered nonpathogenic. Interestingly, the vegetative hyphae and conidia showed normal pigmentation. A peroxisome-associated carnitine acetyltransferase (*CrAT1*) activity was identified as being essential for the appressorial function in *Magnaporthe*. *CrAT1*-minus appressoria showed reduced melanization, but were surprisingly incapable of elaborating penetration pegs. Exogenous addition of excess glucose during infection stage caused partial remediation of the pathogenicity defects in the *CrAT1delete* strain. Moreover, *Mgpex6delete* and *CrAT1delete* mycelia showed weakened cell wall biosynthesis in a glucose-deficient environment leading to appressorial dysfunction in these mutants. Thus, our characterization of a peroxisome biogenesis mutant and an acetyl-CoA transport mutant suggests that peroxisomal beta-oxidation contributes metabolites for melanin and cell wall synthesis during appressorium-mediated host penetration.
These results will be discussed along with our recent data that suggests a possible involvement of Tyrosinases in the pigmentation of vegetative hyphae in *Magnaporthe*.

1145-1345

SYMPOSIUM 39 - *Biosynthetic Gene Clusters for Fungal Secondary Metabolites*

S39IS1 - 0725

The sirodesmin biosynthetic gene cluster of the plant pathogen, *Leptosphaeria maculans*

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Genes responsible for the biosynthesis of secondary metabolites are typically clustered in filamentous fungi. We have cloned a cluster of 18 genes involved in the biosynthesis of an epipolythiodioxopiperazine (ETP) toxin, sirodesmin, from *Leptosphaeria maculans,* which causes blackleg disease of canola. We are analyzing regulation of sirodesmin biosynthesis and its role in disease. Silencing of a Zinc binuclear cluster (Zn2Cys6) gene in the cluster leads to loss of sirodesmin production and decreased transcription of the biosynthetic enzymes. Screening of random insertional mutants for loss of sirodesmin production has led to identification of genes outside the cluster that regulate sirodesmin production. One of these controls biosynthesis of amino acids, which are precursors of sirodesmin.
We are using sirodesmin-deficient mutants to determine the role of sirodesmin in blackleg disease. A mutant in a peptide synthetase, a key enzyme within the cluster, like the mutants in transcriptional regulators described above, does not produce sirodesmin. This mutant makes similar sized lesions on cotyledons to those made by the wild type isolate. However, it colonises stem tissue less effectively than the wild type. A promoter fusion of peptide synthetase with Green Fluorescent Protein has been used to track sirodesmin biosynthesis during growth *in planta*. Transcription of this gene is first detected in hyphae ten days after inoculation of cotyledons. At later stages the gene is transcribed at high levels in pycnidia and during growth in the stem. These findings implicate sirodesmin as a virulence determinant in the late stages of infection of canola when stem cankering occurs.

S39IS2 - 0240

Terrequinone biosynthesis in *Aspergillus nidulans*

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The asterriquinones are a prominent class of fungal secondary metabolites. As they exhibit valuable pharmacological activities, such as antidiabetic or antiviral properties, they have potential in drug lead development. LaeA, a global transcription regulator for secondary metabolism in Aspergilli, was employed for microarray-based screening of the *Aspergillus nidulans* genome to identify actively transcribed natural product genes. Follow-up investigations included gene inactivation, and analytical methods (HPLC, LC/MS, 1D and 2D NMR techniques).
Among the genetic loci found during our screen the genes responsible for biosynthesis of terrequinone A, a member of the asterriquinone class of compounds, were identified, and confirmed by gene inactivations. This is the first report for an asterriquinone gene cluster. Based on these results a generic biosynthetic blueprint for fungal quinoid natural products has emerged. As *A. nidulans* was not known before to produce asterriquinones, we have demonstrated that LaeA represents a powerful mining tool even if the compound is unknown from a given species or if chemical/structural information is unavailable.

S39IS3 - 0401

Fumonisin mycotoxin biosynthesis, genetics and genomics in *Fusarium verticillioides*.

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Fusarium verticillioides is the causal agent of seedling disease, stalk rot and ear rot of maize and can produce the mycotoxins fumonisins. Fumonisins are polyketide derived molecules synthesized through a multi-step biosynthetic pathway by enzymes encoded by a coregulated cluster of genes on chromosome I. Fumonisins are toxic to both humans and animals and have most recently been described as teratogenic, causing neural tube defects in mice. In an effort to reduce or eliminate fumonisin contamination of maize, we are employing genomic resources to elucidate the genetic regulation of fumonisin production. Genomic resources have recently become available for *F. verticillioides* and include libraries of expressed sequence tags (ESTs), microarrays and whole genome sequence. In conjunction with The Institute for Genomic Research (TIGR), we have constructed a dense EST library representing as many as 11,000 unique genes in *F. verticillioides*. This EST library has been utilized to create microarray chips containing oligonucleotide probes for all unique sequences identified from the libraries. Comparison of ESTs generated from different culture conditions has allowed us to identify differentially expressed genes with potential roles in regulating fumonisin biosynthesis. Detailed analysis of ESTs from different culture conditions has revealed previously unidentified genes in the fumonisin biosynthetic gene cluster. In 2005, a 4X coverage of the *Fusarium verticillioides* genome generated at Syngenta and assembled at the Broad Institute was made publicly available. The intersection of whole genome sequence, EST libraries and microarrays is allowing us to more comprehensively define genes and describe their expression at the transcription level. We have combined whole genome sequence to determine the physical location of genes identified from EST analysis with expression data determined from microarray analysis which has allowed us to categorize genes spatially in the genome and their expression temporally.

S39PS1 - 0436

Evolution of polyketide synthase genes in lichenized Ascomycetes

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Fungi synthesize a great variety of secondary metabolites, many of which belong to the structural group of polyketides. Especially lichens, symbiotic fungi living together with green algae or cyanobacteria, produce a large number of polyketides, including several substance classes that are rarely found elsewhere in nature. Difficulties associated with the cultivation of some symbiotic and parasitic fungi have recently sparked an interest in studying the biosynthetic genes of these organisms. The central steps in the polyketide pathway are catalysed by the large enzyme class of polyketide synthases (PKSs). Fungi and - much more infrequently - bacteria possess iterative type I PKSs, monomodular enzymes, which harbour all active sites necessary to catalyse formation of the polyketide. The keto synthase (KS) domain is the most conserved region of the gene and can be used for phylogenetic comparisons of PKSs from a variety of fungal and bacterial genomes. Some fungal genomes contain large numbers of PKS genes, suggesting that gene duplications and horizontal gene transfer between bacteria and fungi are among the evolutionary forces, which have shaped today's diversity of PKS genes in fungi.
In the current study we use KS sequences from bacteria, lichenized and non-lichenized Ascomycota to assess the possible impact of horizontal gene transfer on the evolution of PKS genes in these organisms. Both directions of transfer, from bacteria to fungi and vice versa have been suggested. Here we use a combined Bayesian/maximum likelihood approach to assess the direction of transfer in a statistical framework. Our results suggest that it is not possible to determine the direction of transfer with significant support for every node in the phylogenetic estimate. Inference of the direction of transfer is only possible for a few nodes, if uncertainties of the phylogenetic tree and its branch lengths are accounted for. This shows that great care must be taken with studies claiming horizontal gene transfer without rigorous statistical testing.

1145-1345

SYMPOSIUM 40 - *Biosecurity*

S40IS1 - 0917

The Importance of Mycology in Biosecurity: The NZ Experience

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Biosecurity, the "exclusion, eradication or effective management of risks posed by pests and diseases to the economy, environment and human health" as it is defined in New Zealand, is an area of increasing importance internationally with increasing trade. More recent international trading agreements such as the WTO Agreement on Sanitary and Phytosanitary Trade (SPS Agreement 1994) have focused the management of biosecurity on "technically justified" measures, ensuring that science and research remain the basis of decisions in these areas.
This presentation discusses the importance of mycology in international biosecurity, using a number of past examples were significant fungal-based diseases impacted on a countries economy and environment as illustrations. The presentation then moves to New Zealand's biosecurity programme, using more recent experiences with Phytophthora plant diseases to illustrate how New Zealand responds or intends to respond to such threats through risk management and science research. The presentation concludes by looking at the current gaps in mycological research and potential benefits of international co-ordination in both research and risk management.

S40IS2 - 0796

Biosecurity: latest developments in systems and tools for fungal identification and disease diagnostics

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Early detection, accurate identification and rapid response are key components of an effective plant disease management system for agricultural biosecurity. The detection and accurate identification of a fungal pathogen rely on a strong base of systematic knowledge and the resulting identification tools. Tools include access to published and interactive web-based keys, distance diagnostics capabilities, and an array of molecular and biochemical tests. An effective system also relies on a network of people that include early detectors, trained diagnosticians, and specialized mycologists. The U.S. utilizes a network of personnel for detection and identification of exotic pathogens at its borders. More recently a biosecurity network has been established to identify pathogens within the US. This network utilizes state departments of agriculture and diagnosticians from land-grant universities, which form the National Plant Diagnostic Network (NPDN), in conjunction with scientists in federal laboratories. In both networks distance diagnostics can be useful to make a preliminary and sometimes a final identification, if the fungus is particularly distinctive. In most cases morphological tools are used for a final identification. Increasingly molecular and biochemical tests are being developed and utilized for targeted pathogens and many of these are being adapted for rapid determinations in the field. For some groups of fungal pathogens, such as some ascomycetes and ascomycetous anamorphs, the knowledge base on which to make an accurate identification is lacking. For some groups of fungi an adequate knowledge base exists but rapid access to that information is lacking. Many fungal species have yet to be discovered and thus it is not surprising that a number of recently emerging pathogens must be studied and described as new species. Because these species were not known previously it is impossible to predict their origin and potential damaging effects. Support for systematics is essential to provide the basis for developing tools and tests for accurate identification of fungi.

S40IS3 - 0961

A major exotic disease outbreak, emergency response and eradication: banana black Sigatoka, Tully, Australia, 2001.

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Australia's geographical isolation has assisted its plant industries to remain free of many serious pests and diseases. Strong quarantine measures have been used to support this status. In the past decade, this approach has evolved substantially towards a cohesive national biosecurity system that supports international phytosanitary standards, structured around the shared responsibilities of governments and industries. A good example of the functioning of this system is provided by the response to an incursion of black Sigatoka (*Mycosphaerella fijiensis* Morelet) in Tully in April 2001. The 'hothouse' of this response provided many lessons for the continued development of Australia's plant biosecurity system.

Freedom from black Sigatoka is a benefit the Australian banana industry values greatly. Fungicide and labour inputs are far lower than would be required if this disease was present. The industry, producing 300 000T per year worth over $300 million, is based primarily in tropical far north Queensland. Black Sigatoka is endemic nearby in the Torres Strait and Papua New Guinea. Since 1981, as part of a specific black Sigatoka biosecurity program, nine outbreaks were detected and eradicated on Cape York Peninsula in remote areas and in one case in an isolated commercial plantation. Detection of black Sigatoka in Tully, the heart of the 9 000 ha industry, came as a great shock. Industry's concern about the expected yield losses and control costs was exacerbated by strict market access restrictions that were placed immediately on fruit from a 50 km radius around infected areas. An emergency response was mounted, based on an incursion management plan and the national system for managing exotic pest incursions. Initially, infected fields were destroyed, with voluntary compensation paid by the industry. As this became unaffordable, the approach changed to a zero-disease standard to be achieved by removal of diseased leaf tissue, a compulsory area-wide fungicide program and intensive surveillance. Over 13 000 feral and unmanaged residential plants were killed. Industry and community participation was exceptional, although regulatory enforcement was available when required. A PCR-based molecular diagnostic assay was developed and was of great assistance. Disease was found in 25 sites in over 8 900 diagnostic samples. Efforts were assisted by the occurrence of two dry years. Eradication was completed in May 2002. Area freedom surveillance was conducted, with over 6 300 samples examined leading to a declaration of pest freedom in March 2005. Full market access was restored and early warning surveillance resumed. This paper will use the response to black Sigatoka in Tully and other examples to illustrate the functioning of the emergency plant pest response system in Australia.

S40PS1 - 0484

The utility and limitations of positive and negative controls for PCR detection of quarantine pathogens.

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Species-specific PCR test methods are increasingly being developed for the detection of plant pathogens of quarantine importance. The possibility of false negatives, and their significance for bio-security measures, is an on-going concern with the application of this technology, particularly as PCR is commonly prone to failure due to enzyme inhibition or operator error. Incorporation of Internal Amplification Controls (IACs) can increase confidence in negative test results by demonstrating the success of each PCR reaction, but IACs alone may be insufficient to prevent false negative results. Here we describe the development of IACs for PCR detection of *Puccinia psidii*, a pathogen with potential devastating consequences for Australian biodiversity if introduced, and discuss the need for additional controls.

IAC plasmids were constructed by cloning PCR products generated using 'hybrid' primers. The 5' end of each 'hybrid' primer comprised a *P. psidii*-specific primer with an additional 3' portion complementary to an appropriate region of plasmid pUC18.

A 1200bp fragment was successfully amplified from recombinant plasmids p04L1-6 and p04L2-4 using the *P. psidii* nested PCR diagnostic primers P1/P6 and P2/P4. PCR product was detectable from an IAC template concentration of 0.1 fg/Ìl, but the addition of plant or fungal DNA necessitated an increase in IAC concentration to 1 fg/Ìl. A high concentration of *P. psidii* template DNA competitively inhibited the amplification of the larger product from the IAC.

The inclusion of an IAC can increase confidence in negative test results, but may also be misleading. In addition to the inclusion of IAC, DNA amplification quality should be demonstrated by amplification with non-specific primers as degradation of DNA before or after extraction or accidental loss of DNA during the purification process will give a false negative that is not detected by the addition of an extraneous IAC. The advantages of exogenous rather than endogenous IACs for *P. psidii* is also discussed. In addition to positive controls, the requirements for appropriate negative controls are discussed.

Perhaps this could be widened to include reasons for the inhibition, as t5his is what we are continually trying to avoid with PCR for such purposes. Standardisation of DNBA extraction and post extraction clean up is an issue. Rember, more and more folk are accepting that a test method is more than just the primers, but also a complimentary, relevant DNA extraction procedure (and to that end a sampling approach….).

S40PS2 - 0634

Dissemination of aerial and soilborne Phytophthoras by human vectors

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Two new invasive Phytophthora pathogens, *Phytophthora kernoviae* and *P. ramorum*, have recently established in the UK. They are most prevalent in the south west of England in woodland areas where they cause intense episodes of dieback on wild rhododendrons (*Rhododendron ponticum*), but also cause lethal stem cankers on a range of broadleaf trees. As both these Phytophthoras are aerial pathogens their deciduous sporangia, produced on foliage of infected rhododendron and other foliar hosts, are dispersed by wind and rain splash on a local basis. However, patterns of disease spread suggest that vertebrate vectors may also aid the spread of these pathogens over longer distances. Infected rhododendron leaves are quickly shed and incorporated into the dense litter layer and people and animals frequently walk through these contaminated areas. To assess the likelihood of walkers picking up infested soil or litter on their feet, a study was set up to analyse how frequently *Phytophthora* could be isolated from the soil or litter attached to people's boots, particularly those walking in known *P. kernoviae*/*P. ramorum* woodlands and gardens. The study started in July 2004 and has continued through 2005 and 2006. As the aim of the study was to determine (1) which species and (2) how frequently viable Phytophthora inoculum was moved by human vectors, so baiting methods were employed for isolation and detection. Several different species of *Phytophthora* have been isolated, and more than 30% of samples collected from walker's boots were contaminated with *Phytophthora*. The most commonly occurring species was *P. citricola*, but 10-15% of the samples contained either *P. ramorum* or *P. kernoviae*. Other Phytophthoras found include *P. ilicis* (another aerial Phytophthora), and also *P. cambivora*, *P. cryptogea*, *P. gonapodyides* and a single finding of *P. hibernalis*. It has yet to be established if the amount of Phytophthora inoculum carried on boots can initiate a new infection focus in an area remote from the source of *Phytophthora* inoculum, but it is clear from this study that human vectors could provide significant pathways for disease spread for quarantine pathogens such as *P. ramorum* and *P. kernoviae* as well as other aerial and soilborne Phytophthoras.

POSTER SESSION 4: CELL BIOLOGY AND PHYSIOLOGY

PS4-386-0019

Becoming less protected;germination of highly stress-resistant ascospores of *Talaromyces macrosporus* includes unique cellular features.

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Ascospores of *Talaromyces macrosporus* are the most resilient eucaryotic structures described to date. They survive heat (above 85 °C), high pressure, and drought. Besides, they show constitutive dormancy and are triggered to germinate by a heat treatment at 85 °C. This contribution addresses activation of the spores to germination and the changes inside the cell during early germination. Upon heat activation changes in the cell wall were observed with different techniques. For example, fluorescence microscopy indicated a change in the permeability of the cell wall. Electron paramagnetic resonance studies showed that the viscosity inside the cells is extremely high. Early germination was characterised by low respiration, trehalose degradation and glucose release, but high viscosity remained. Then, a sudden ejection of the inner cell through the outer, very thick, ascospore cell wall was observed. The latter process was dubbed prosilition. Cryo-planing and scanning electron microscopy exhibited a connection between the ejected cell and the emptied outer cell wall. Prosilition is thought to be important for renormalisation of the spore while respiration increased strongly and cell viscosity dropped to a normal level.
Activation by high temperature was associated with the release of large amounts of a small protein from the cell (wall). This protein was estimated as being the single dominant protein of ascospores and responsible for 5% or more of the amount of protein of these cells. After terminal amino acid characterisation, we constructed a degenerative primer and after (RACE)-PCR, the sequence of a small protein (called PLAY) was obtained and it contained one short intron. The genome of *Talaromyces macrosporus* contained one copy of this gene and its expression as judged by Northern blotting was strictly related to the formation of fruit bodies (that contain ascospores). Further research will be done to evaluate its role in dormancy and heat-resistance.
Knowledge on the behaviour of these highly stress-resistant cell is related to protection mechanisms of biological compounds and the identification of novel cell wall components (proteins). Furthermore, the ascospore is still a relatively scarcely studied, but abundantly present fungal structure and its study might reveal new basic principles of fungal biology.

PS4-388-0033

Extracellular enzymes from thermophilic fungi

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Thermophilic fungi comprise a small ecological group defined solely on temperature requirement for growth, ranging between 200C to ≥ 500C. There are about 40 known species of fungi, which have been characterized. Many bacteria and Archaea from thermal habitats have been explored for the industry, and have provided it with the thermoactive enzymes. In contrast fungal enzymes are much more acid tolerant than the bacterial enzyme which is an important factor for many industries. In the present investigation 29 species of thermophilic and thermotolerant fungi belonging to 14 genera were isolated from north Indian soils. These species were screened for their growth characteristics and ability to produce extracellular amylase, exo-glucanase, endo-glucanase, b-glucosidase, lipase, and xylanase. Of these 29, 4 species (*Chaetomium thermophile*, *Thermomyces lanuginosus, Malbranchea sulfurea* and *Scytalidium thermophilum*) were selected to study enzyme production using different substrates. *M. sulfurea* exhibited maximum amylase activity (0.22±0.005 U ml-1). Wheat straw (WS) and alkali treated wheat straw (AWS) was used to compare the production of exo-glucanase, endo-glucanase, and b-glucosidase. *S. thermophilum* produced highest exo-glucanase activity, 259.4±0.5 IU ml-1 and 148±5.7 IU ml-1 on WS and AWS respectively. Highest endo-glucanase activity was also shown by *S. thermophilum* being 230.2±1.7 IU ml-1 and 175.1±1.5 IU ml-1 on WS and AWS respectively. However, b-glucosidase production was highest in the culture filtrates of *C. thermophile*. Similarly maximum lipase activity was observed in the culture filtrates of *M. sulfurea* (92.0±8.1 IU ml-1). Maximum xylanase activity was shown by *C. thermophile* and *M. sulfurea* on xylose containing media being 0.82±0.14 IU ml-1 and 0.81±0.11 IU ml-1 respectively. In the present investigation, it was realized 'Do fungal growth profiles correlate with enzyme characteristics?' This question is of interest both from academic and industrial point of view and can be enlighted with thermorelated studies.

PS4-389-0042

Mechanical disruption of *Candida* cells for protein estimation and enzymatic activity

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The outermost covering of a cell is broken down to release protoplasmic components for examination or analyses. Such cell lysis procedures are employed in studies related to molecular basis of disease, host-pathogen interaction, biotechnology, enzymology, among others. Due to the complexity of their cell wall composition, some effort is usually required to disrupt the cell wall of yeasts. *Candida* species are medically important yeasts which cause opportunistic infections in man. Studies on *Candida* are often based on molecular approach and enzyme production which require protein extraction from disrupted cells. This study was undertaken to compare the efficacy of three cell lysis methods with respect to amount of recoverable crude protein and enzymatic activity.
Using Glass beads, Sonicator and French pressure cell press separately, cells of ten clinical *Candida* strains harvested from cultures with same growth conditions (broth cultures in Yeast Peptone Dextrose incubated for 48 hours under aeration at 30 degrees Centigrade) were lysed, following standard protocols. The efficacy of each method was determined by the protein concentration and the activity of an endoproteinase (Protease A) in each lysate.
Protein concentration values for French press were higher than for Sonicator and Glass beads (mean = 5.6, 4.0 and 0.6µg/ml, respectively). Enzymatic activity for French press and Sonicator methods were comparable and about seven-fold that of Glass beads method.
It is clear that Glass beads method which is the cheapest and most commonly used is the least efficient. The results have indicated that French pressure cell press is the most efficient of the three methods. French press is an expensive equipment to acquire but has a high efficiency in extracting crude protein. The Sonicator, if properly used might be as efficient as the French press.

PS4-390-0051

Saprotrophic fungi transform organic phosphorus from spruce litter needles

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Phosphorus (P) transformation from spruce (*Picea abies*) litter needles and their decomposition were simulated in systems with single strains of autochthonous saprotrophic fungi and their mixtures. Needle colonizing basidiomycetes were represented with one strain of *Setulipes* (*Marasmius*) *androsaceus*; ascomycetes were represented with strains of *Chalara longipes*, *Ceuthospora pinastri*, *Mollisia minutella*, *Scleroconidioma sphagnicola* and unknown ascomycete NK11. Systems were incubated for 5.5 months after inoculation. Fungal colonization of litter needles was confirmed after reisolation test. P transformation was determined by solution 31P NMR analysis of alkaline extracts from needles. The degree of litter decomposition was estimated from the decrease of C/N ratio. Fungal colonization resulted in production of phosphonates, diphosphates and polyphosphates in the majority of fungal strains accompanied by synchronous loss of the phosphate monoesters from needles. All these changes may be directly attributed to the fungal activity as sample needles collected from the systems and placed on agar media produced mycelia of inoculated strains. However, no regular pattern was observed for studied strains. Ascomycetous strains were generally less effective in phosphonates and polyphosphates production and C/N decrease than the strain of *S. androsaceus*, causing substantial C/N decrease from 25.8 to 11.3. *S. androsaceus* dominated in competition being reisolated with the highest frequency from mixture systems followed by *S. sphagnicola*. These results confirmed our previous findings about competition of these fungal strains paired on agar plates with low nutrient agar media. *S. androsaceus* was able to replace other litter colonizers. On the other hand, mycelium of *S. sphagnicola* stayed viable although being completely replaced suggesting its ability to withstand stress caused by competition. Competition among fungal strains inhibited the decomposing activity as the C/N in mixture systems reached 21.8 and production of phosphonates and polyphospates decreased. We suggest that production of polyphosphates by *S. androsaceus* may substantially contribute to the P cycle in forest ecosystem as this fungus belongs to the most frequent decomposers of coniferous litter needles.

PS4-391-0052

Biochemical screening of some members of Xylariaceae

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The Xylariaceae is a large and well-known family of the Ascomycotina. Its members are world wide in their distribution however in general this family is well represented in tropics. The family has been extensively investigated with regards to secondary metabolites. Secondary metabolites produced in liquid culture have been used as an aid to establish a satisfactory systematic arrangement.

In India, Western Ghats of Maharashtra is a very rich area for the members of Xylariaceae. The reported work deals with species of *Hypoxylon*, *Xyalria* and *Nemania* collected around Monsoon season from various localities of the forests. These were identified with the help of traditional morphological characters using relevant literature. *Xylaria* is found to be the dominant genera in India. The identified species were subjected to culture on malt extract agar medium for obtaining progressive cultures that lead to production of secondary metabolites in liquid medium. Succinic acid derivatives are the most frequently isolated metabolites till today. The screening of metabolites was based upon chromatographic techniques for separation of major compounds and spectroscopic analysis for structure determination and identification of the isolated metabolites. Since majority members of Xylariaceae are wood habitants, selected enzymatic activities were also investigated. The details of methodology of extraction, separation, purification and identification of compounds will be discussed during the poster presentation.

PS4-393-0076

Effect of the Medium pH and Cultivation Period on Mycelial Biomass, Polysaccharide and Ligninolytic Enzymes Production by *Ganoderma lucidum*

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The fruiting bodies and mycelium of *Ganoderma lucidum* contain polysaccharide, which have immunomodulating effects and some of them inhibit the growth of several cancer cells. In chemical structure the polysaccharide are highly branched 1,3-b-D-glucans which degree of branching is in correlation with their immunomodulating effects. The aims of this research were determination of the optimal medium initial pH for biomass, polysaccharide, and ligninolytic enzymes production, as well as the study of dynamic of their synthesis.
The effect of medium initial pH to the biomass, extracellular and intracellular polysaccharide, and ligninolytic enzymes production by *Ganoderma lucidum* was investigated at the different initial pH (2.0, 3.0, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0) of the synthetic medium after 7 and 14 days of cultivation. The influence of cultivation period was studied at the optimal pH for biomass production and measurements were done from the 4th to the 13th day of cultivation. The mycelial biomass was measured after separation by centrifugation and drying. Content of extracellular polysaccharide was determined by precipitation of crude supernatant by 95% ethanol at 4∞C during the night, separation by centrifugation, and drying at 50∞C. Amount of intracellular polysaccharide was studied by macerating and cooking of dry, frozen mycelium in distilled water at 100∞C for one hour and then the method was the same as for extracellular polysaccharide. Laccase, Mn-depending peroxidase, and versatile peroxidase activities were determined spectrophotometrically.
The maximal biomass production was recorded at pH 4.5 after 7 days (8.80 gl-1 of the medium) and pH 5.0 after 14 days of cultivation (20.60 gl-1 of the medium). The maximal production of extracellular polysaccharide was obtained at pH 7.0 (2.48 mgml-1 of supernatant) and pH 3.0 (5.20 mgml-1 of supernatant), while pick of intracellular polysaccharide synthesis was at pH 7.0 (69.44 mgg-1 of dry weight) and pH 5.5 (69.93 mgg-1 of dry weight) on the 7th and on the 14th day of cultivation, respectively. The ligninolytic enzymes were not produced at any pH of the medium, which can be explained by fact that composition of used medium was not suitable for the enzymes production by analysed *G. lucidum* strain.
The maximum of biomass production was obtained on the 11th (23.40 gl-1 of the medium), of extracellular polysaccharide on the 7th (1.60 mgml-1 of supernatant), and of intracellular polysaccharide on the 6th (52.84 mgg-1 of dry weight) and on the 10th day (55.46 mgg-1 of dry weight) of cultivation.

PS4-394-0077

Structural features in haloalkalitolerant ascomycete *Heleococcum alkalinum* Bilanenko et Ivanova responsible for its adaptation to extreme growth conditions

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Heleococcum alkalinum Bilanenko et Ivanova (Hypocreales) is recently described haloalkalitolerant ascomycete which was isolated from saline soda soils (pH 10-11) of Central Asia and Africa.
It was cultivated on alkaline agar media (pH 10-10,5) which is found to be most suitable for the species.
Morphological, cytological and ultrastructural studies revealed some unusual particular features which were found to be of adaptation to extreme conditions.
Most interesting structures were special vacuoles present predominantly in conidia, phialides and aerial mycelium. Such vacuoles differ significantly from usual vacuoles by the tonoplast structure. They were found to play an important role in osmoregulation under extreme growth conditions accumulating Cl- in cultures grown on media, containing 400 mM NaCl. The vacuoles in conidia fused into one single vacuole and increased in size in response to osmotic shock and recover their structure in isotonic solution. Such a way they were defined to be main salt-accumulating organs in *H. alkalinum*.
Chondriom structure was also observed to change according to variations of environmental conditions. We observed gradual fragmentation of mitochondria in response to osmotic shock (increase of NaCl concentration to 1-4M in surrounding solution). Although stained by rhodamine, all the chondriom maintained high fluorescence intensity. Moreover, even in 4M NaCl no plasmolysis was observed. So we suppose that such chondriom behavior is also an adaptive reaction.
In cultures grown on standard medium (malt extract agar) where a lot of modified mycelium was observed, most part of chondriom fell to fluoresce. TEM data revealed that most of mitochondria in that case were of abnormal size or structure, which demonstrate its functional faults.
In addition, ascomal centrum development in *H. alkalinum* greatly differed from other species, which is also thought to be caused by extreme growth conditions.
More common features were observed along with that mentioned above. sThe list includes thick cell walls in ascospores and multilayered ascomal peridium with thick cell walls containing large amounts of brown pigment.

PS4-395-0086

Purification and characterization of an extracellular serine protease serving as the potential pathogenic factor in *Clonostachys rosea*

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The fungus *Clonostachys rosea* (syn. *Gliocladium roseum*) is a common saprophyte in the soil and it has been reported to be toxic to nematodes such as *Bursaphelenchus xylophilus*. Since the nematode cuticle is a flexible exoskeleton composed primarily of proteins, the involvement of protease in the infection should be reasonable. In our study, an extracellular protease (PrC) was purified to homogeneity from an isolate of *C. rosea* by the methods of precipitation with ammonium sulfate, hydrophobic interaction chromatography and anion-exchange chromatography successively. The nematicidal activity of PrC was confirmed the fact that 80±5% of nematodes could be immobilized and degraded after treating with PrC for 48h. The purified protease had a molecular mass of 33kDa and displayed optimal activity at 60 ?, pH 9-10. Furthermore, the protease PrC hydrolyzed a broad range of substrates including casein, gelatin and the purified nematode cuticle. The sequencing of N-terminal amino acid residues revealed the protease of PrC should belong to serine protease family, which was consistent with the analysis of the protease inhibitors. The multiple sequences alignments demonstrated that the purified PrC shared 32-80% homology with the known cuticle-degrading protease from nematophagous or entomopathogenic fungi *Vercillium lecanii*, *Trichoderma harizianum*, *Metarhizium anisopliae*, *Athobotrys. oligospora*, *Pochonia chamydosporia*, *Lecanicillium psalliotae*, *Paecilomyces lilacinus*, implying PrC play a role in the infection against nematodes. Compared with the other serine proteases, it was worth noticing that either biochemical characterizations or amino acid sequences of PrC showed high similarity to VCP1, P32 pSP3, and Ver112, which were isolated from egg-parasitic or endoparasitic fungi *P. chlamydosporia*, *P. suchlasporia*, *P. lilacinus* and *L. psalliotae*, respectively. However, PII and Aoz1, another tow serine proteases isolated from nematode-trapping fungus *A. oligospora*, had the lower p*I* and higher molecular mass in biochemical properties. The homologeous analysis based on amino acid sequences suggested that all of the serine proteases mentioned above should be divided by two main clusters; but PII and Aoz1, by contrast with those from egg-parasitic or endoparasitic fungi including PrC, were placed in the different subfamily.

PS4-396-0087

Water availability and metabolomic profiles of *Epicoccum nigrum* and *Sarophorum palmicola* grown in solid substrate fermentation systems

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There has been interest in using environmental screening procedures for enhancing the metabolomic production profiles by fungi which are of pharmaceutical interest. Surprisingly few attempts have been made to examine the ecological context of isolated species to improve the potential for maximising metabolomic profiles and specific metabolites based on a more effective eco-environmental approach.
This study examined two ecologically distinct species, *Epicoccum nigrum* and *Sarophorum palmicola*, in solid substrate fermentation systems over a range of water availability conditions (water activity, aw). Total secondary metabolite profiles were obtained by HPLC + diode array detection after 14 days incubation on cereal-based substrates in relation to four aw treatments.
Temporal studies (24 d, *E. nigrum* and 18 d, *S. palmicola*) showed that metabolite production profiles varied markedly between the two fungi. For *E. nigrum*, metabolite production generally increased with reduction in aw, and was optimal in the range 0.99 – 0.97. In contrast to this, for *S. palmicola*, metabolite production was restricted to the highest aw level used, 0.998, and declined to zero at 0.99 aw. Statistical analysis revealed that time, aw and their interactions were significant in all cases ($p<0.001$).
Using a more eco-environmentally relevant screening system has demonstrated that the types and range of metabolites produced by fungi can be significantly enhanced. The use of ecophysiological stress approach, when combined with effective statistical designs can be effectively utilised to significantly enhancing natural product discovery.

PS4-397-0106

Diversity of agarics on elephant dung

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Compared to other groups of fungi, basidiomycetes, with the exception of the "Coprini" and a few related genera, are rather rarely seen on dung. This is because dung, being an ephemeral substratum in most cases, cannot support long-life-cycled and large-fruit-bodied basidiomycetes. Elephant dung is an exception here because of the following reasons: droppings are comparatively more massive, are composed mostly of lignocelluloses, and it takes almost a year before they are fully disintegrated. These features of elephant dung favour colonization and development of agarics. In the literature, however, only very few agarics have been reported to grow on elephant dung. During our studies spanning past several years, we have found several agarics growing on elephant dung. An exclusive account of the agarics (except the "Coprini") associated with elephant dung is presented here. Agarics were collected from dung of both wild and domesticated elephants. Conventional mycological techniques for examination of agaric specimens were used in the study. Nineteen species belonging to twelve genera representing six agaric families were found associated with elephant dung and are documented here along with a key to the species. The agarics are: *Macrocybe gigantea*, *Entoloma anamikum*, *Volvariella volvacea*, *Stropharia bicolor*, *Stropharia rugosoannulata*, *Psilocybe subaeruginascens*, *Psilocybe subcubensis*, *Psilocybe pegleriana*, *Psilocybe coprophila*, aff. *Panaeolina rhombisperma*, *Copelandia cyanescens*, *Panaeolus antillarum*, *Panaeolus rickenii*, *Bolbitius coprophilus*, *Agrocybe guruvayoorensis*, *Pholiotina indica*, *Conocybe volvata*, *Conocybe brunneoaurantiaca* and *Conocybe pseudopubescens*. The following five species encountered in the present study are known to grow only on elephant dung: *Agrocybe guruvayoorensis, Conocybe volvata, Conocybe pseudopubescens, Pholiotina indica, Stropharia bicolor*. The most remarkable outcome of the present study is the recognition that elephant dung, a restricted and transient ecological niche, is capable of supporting such a large number of agarics. As far as we know, this is the first comprehensive account in the whole world on agarics associated with elephant dung.

PS4-397a-0116

A Qualitative Investigation On Tea Processing Houses Air Fungal Pollution In The North Of Iran , Gilan Province Estern Region

leila Modiri, Arash Chaichi-Nosraty, Mohammad Faezi Ghasemi, Ali Reza Khosravi, Ali Reza Massiha, Shiva Roofigary Haghighat
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Fungi Are Obiquitous And Infact Account For At Least 25% Of The Earth's Biomass So Abundant In Many Area Or Spaces Serving Optimum Temprature And Humidity .
Exposure To Air Borne Fungi Mmay Be Associated With Health Hazards Rangeing Non – Specific Irritation To Inflamative Infections And Tends To Become A Significant Health Risk To An Increasing Number Of Workers In Various Occupations Throughout The Nations .
Thus , The Potential Problematic Outcomes Have Led To A New Legal Industry With Vastating Impact On The Immune Insurance Versus Discomforts , Based On A Standard Classification Establishment For Indoor Climate Hygiene .
Realy , Should Be Noted That The Importance Taxa Identified Is Much More Imporatant That The Absolute Number Of Colony – Forming Units , Allowing Comparison Among Indoor Versus Outdoor Genera Reflecting Distinct Conditions .
In This Respects Totally 31 Typical Common Tea Processing Houses Dust Bioaerosols Were Sampelified And 1235 Mold Colonies Were Isolated Due To Direct Microscopy And Culture - Based Inspective Conventional Mycologic Methods , During May To August 2005 . Finaly 20 Different Genera Of Habitate Fungi Were Collected And Confirmed From 207 Conducted Plates . Of Defined Geographic Location , This Is The Largest Study Of Airborne Indoor Fungal Species With A Rutine Procol To Date .
There Are No Ageement Of Any Standardized Protocol To Evaluate Distinctive Indoor Fungi Populations And Resulting Health Complications So Significance Of Hygiene Reports Seem Dubtfully .
To Clarify Indoor Mold Measurments , Colonization Versus Contamination , Upon Hygien Survay Highlighting Scenarios And Covering Substantial Damages Associated To Fungi , Further Researches Is Needed For New Legal Verification .

PS4-398-0143

Stachylina in India: occurrence and relationships with temperature and hydrogen-ion concentration of the waters

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Bioinventoring of Harpellales (Trichomycetes) was undertaken to fill the lacuna in our knowledge of these fungi from India.
Bloodworms *(Chironomus* sp, Chironomidae) were regularly collected for a year (Jan-Dec. 2004) from stagnant water bodies in and around Lucknow by scooping the muddy water using iron strainer. Temperature and pH of the water were recorded at the time of collection of bloodworms. The bloodworms were dissected in a drop of distilled water under binocular stereomicroscope and peritrophic membranes were examined under phase contrast microscope in water mount for the presence of the thalli of *Stachylina* on them.
Stachylina chironomidarum and *St. grandispora* were observed. *St. chironomidarum* was not common in occurrence while *St. grandispora* was found throughout the year, of course, in varying percentages as shown in table below. *St. grandispora* exhibited enough variations in the number and size of the trichospores.
Per cent occurrence of *Stachylina grandispora* in the Bloodworms collected in different months from stagnant water bodies in and around Lucknow (Jan. 2004-Dec. 2004)

Month	Water temperature in Celcius.	PH	Number of Bloodworms collected	% occurrence of *Stachylina grandispora*
January	19	8.00	09	45
February	23	8.00	10	59
March	29	9.00	08	55
April	30	9.10	13	36
May	32	9.00	09	34
June	32	9.20	13	45
July	27	9.00	12	46
August	29	9.00	04	38
September	29	8.00	07	35
October	26	8.00	09	70
November	18	8.00	07	29
December	11	8.00	06	23

The correlation coefficient (r) analysis of the present set of data is not significant but the relationships do exist.

PS4-399-0218

Fungal Ligninolytic Enzymes in the Forest Soil Environment: Occurrence, Distribution and Role in Soil Organic Matter Transformation

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Ligninolytic oxidases and peroxidases of saprotrophic fungi are the enzymes responsible for the transformation of lignin – the second most abundant biopolymer. In forest soil, ligninolytic enzymes contribute to the degradation of lignin in decaying leaf litter and to the transformation of humic substances with a similar chemical structure. The aim of this work was to quantify the activity of ligninolytic enzymes found in oak forest soil with respect to their spatial distribution and temporal variability, to find its producers, litter decomposing fungi (LDF), and to identify the role of the enzymes in the transformation of soil organic matter.
Enzyme activity was measured in environmental samples from oak (*Quercus petraea*) forest and linked with fungal occurrence and biomass and the production of other extracellular enzymes. The species producing ligninolytic enzymes were isolated from the studied soil and tested for their ability to perform degradation of freshly fallen leaves and to transform humic substances isolated from the site.
Laccase and Mn-peroxidase (MnP) but not lignin peroxidase were found in the studied soil with laccase activity being by far higher. Activity of both enzymes decreased with the soil depth and showed a patchy pattern of horizontal distribution with "hotspots". These were in case of laccase often associated with the occurrence of LDF fruit bodies. During the growth of LDF isolates on oak leaves, laccase was again the major enzyme and it was produced mainly in the initial phases of decay. Ligninolytic enzymes also contributed to the transformation of humic compounds. Their production was usually induced by humic acids, but humic and fulvic acids also competitively inhibited laccase activity.
Laccase and MnP play important roles in the turnover of carbon in the soil environment during the transformation of lignin in the fresh biomass (fallen litter) and nutrients liberation from the recalcitrant humic material.

PS4-400-0223

Gene expression during the switch from saprotrophic to pathogenic phases of growth in the root and butt rot fungi *Heterobasidion annosum*

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The tree pathogen *Heterobasidion annosum* can prevail in dead roots and spread from dead tissue to living trees. We therefore examined whether a shift in gene expression occurs during the switch from saprotrophic to pathogenic growth. We used a macro-array differential gene analysis to identify genes that were either induced or suppressed during either stages of growth of the fungus. Macro-arrays containing a selected number of clones from cDNA library of *H.annosum* and *H. parviporum* representing a functionally diverse range of genes were investigated. Dead pine seedlings were inoculated with *H. annosum* and transferred to water agar plates containing living pine seedlings, the hyphae were then sampled from various stages of interaction before and after contact with the pine host. Total RNA was isolated, amplified to aRNA and used as probes for differential screening of the macro-array membranes. Signal intensity values for differentially expressed genes was documented with Quantity one (Bio-RAD) and the data was statistically analysed to identify significantly differentially expressed genes. A clear shift did occur in gene expression between saprotrophic and pathogenic growth. Several clones with homologues to known pathogenicity factors were differentially expressed, among them a clone with a pathogenicity MAP Kinase homologue.

PS4-401-0234

Hybridization and heteroploidy as sources of biodiversity in filamentous fungi.

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In fungi, a form of an interspecific hybrid is a dikaryon from parents belonging to different species. In sexual interaction, the male and female structures interact to generate a dikaryotic condition. Hybrids may maintain the dikaryotic state, or they may undergo karyogamy and normal meiosis to reconstitute the euploid state, or they may undergo abnormal meiosis to yield a heteroploid hybrid. Data about heteroploidy are controversial. We have started to compile a Fungal Genome Size Database http://www.zbi.ee/fungal-genomesize/, which at present consists of more than 1000 records. Employing electrophoretic karyotyping and studying mostly asexual fungi, it has been found that variability in genome size of a fungal species is a rule rather than an exception (Beadle 2003). At the same time, using cytofluorometric investigation of sexual fungi the stability of genome size among different strain and species was demonstrated.
What happens to the size of a hybrid genome during meiosis can be seen by analyzing DNA content of a spore print of a single fruit body.
Our studies using flow cytometry have revealed that spore prints of *Pleurotus, Lentinula, Phellinus* and *Cystoderma* species may represent spore populations with two different genome sizes. Divergence was even more evident in a spore print of an industrially cultivated strain of *P. ostreatus* (Kullman 2002). Fruit bodies of *Pleurotus ? Lentinula* strains obtained by artificial mating produced two distinct spore populations whose genome sizes were comparable to those of their parental strains (Kullman et al. 2005).
We studied 26 specimens of *P. ostreatus, P. pulmonarius and L. edodes.* Genome sizes of *P. pulmonarius* and *L. edodes* differ but specimens of *P. ostreatus* may produce two distinct spore populations whose genome sizes were comparable to *P. pulmonarius* and *L. edodes.* Fruit body of *P. ostreatus* may contain nuclei of two distinct species. Such a fungus is a hybrid possibly supporting the opinion of the ongoing speciation in that complex.
Our results seem to confirm that parental genomes of different sizes segregate in meiosis. A fungus having divergent spore populations had characteristics intermediate between *P. ostreatus* and *P. pulmonarius*. We presume that divergence that arises from spore print reflects the fate of a hybrid genome in meiosis (Kullman 2000 http://www.ut.ee/ial5/fce/folia.html, 2002).
Our Fungal Genome Size Database demonstrates that the intraspecific variety of *P. ostreatus* genome remains within the same limits according to literature data and our results. The difference in chromosome number and genome size appears as aneuploidy and heteroploidy.
The research was supported partly from the German Academic Exchange Service (DAAD) research grant A/98/07170 and by the grant No. 4989 of the Estonian Science Foundation.

PS4-402-0238

Lectin Accumulation in Edible Wild Mushrooms in Northeastern Thailand

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Fungal lectins, the diverse multivalent carbohydrate-binding proteins of non-immune origin, have been becoming more interest due to the discovery of some of the lectins exhibiting antitumor activity as well as other potential activities such as mitogenic, immunoenhancing, and vasorelaxing activities. Some lectins derived from plants, are currently employed in a number of biomedical and clinical research. In Thailand, the great diversity of edible wild mushroom species has been reported. These mushrooms could provide an alternative source of lectins. In this study, the accumulation of lectins in fruit bodies of edible wild mushrooms found in Northeastern Thailand, was investigated. Some edible mushroom lectins may have cytotoxic activities against human cancer cells.
Fresh fruit bodies of edible wild mushrooms were collected from their natural habitats and from local markets in Northeastern Thailand. The mushroom specimens were classified and identified using conventional methods based on their morphological characteristics, then dried, and ground into powder. Crude lectins were extracted from the powder, and detected their unique properties by hemagglutination assay using red blood cells from various animals (goose, guinea pig, mouse, rabbit, rat, and sheep). The extracts accumulating high lectin titers were selected to test for their temperature stability and cytotoxic activities against cancer cell lines, human epidermoid carcinoma (KB) and human cervical carcinoma (HeLa).
From the 2-year collection of edible wild mushroom specimens, a total of 88 specimens with high morphological variation and belonging to the family Russulaceae, the dominant family found, were selected for crude lectin extraction. Sixty one specimens exhibited the incidence of lectin accumulation in their fruit bodies. The lectin extracts predominantly agglutinated rabbit and goose red blood cells, and performed their unique lectin properties depending on the mushroom strains. Crude extracts of six edible mushrooms in the genus *Russula* displaying their high lectin titres, were stable at 4 and 30ºC for 24 h, and at 60-70ºC for 30 min. Three of the six extracts still contained almost 50% of lectin properties after exposing to 90ºC for 30 min. The six extracts exhibited different cytotoxic activities against KB and HeLa cell lines with IC50 values ranging from 16 to 170 and 16 to 700 mg/ml respectively. IC50 values less than 30 mg/ml were designated as cytotoxicity.
Specific strains of edible wild mushrooms in the family Russulaceae, particularly in the genus *Russula*, found in Northeastern Thailand, accumulated lectins in their fruit bodies. Some of the lectins showed their stability at high temperature, and could remain after cooking. Some also had cytotoxic activities against cancer, KB and HeLa, cell lines, which will be useful for further investigation.

PS4-403-0239

Estimation of fungal genome size using DAPI- image cytometry

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In providing quantitative data of nuclear DNA for the purpose of fungal taxonomy, photometric cytometry (PC) have played an important role. Fluorescence microscopy combined with computerised image analysis, i.e. image cytometry (IC), offers an alternative tool for assessing genome size. These techniques allow direct visualization of hyphae and simultaneous measurement of nuclear fluorescence intensity. We developed a simple method for quantitative evaluation of nuclear DNA in fungi using DAPI-IC. The intensity of signals from individual nuclei was quantitatively measured in digitized images. This simple IC performed on fruitbodies or on pure culture preparations enables to detect the amount of nuclear DNA in fungal cells.
Staining Protocol . A slice of a fruitbody or a hypha of a pure culture were fixed in Carnoy's solution and stored at 4 °C until used, or at least for 1 h. To stain DNA, the fixed material was slightly dried and incubated with 0.5% Pepsin pH 1.8 for 7 min at room temperature by slow shaking. Next, the fourfold volume of DAPI at 2˜g ml-1 TRIS buffer was added, and the sample was incubated for 45 min by slow shaking. Then the slices were placed in a drop of glycerin on glass slides, minced and rinsed gently with a shaving blade and squashed under the cover slips. The slides were stored at -20 °C. For one experiment, the slides of all specimens were prepared and analysed during one measurement session.
Processing with Image Pro Plus 4.5 (manufactured by Media Cybernetics, USA)
Hyphal nuclei were observed under 40x and 20x Olympus LCPlan FL objectives and the images of the nuclei in the areas with low background noise were saved on the hard disk of the computer as TIFF files. Image-Pro Plus 4.5 was used to grab and process the images with local background determination: i.e. the nucleus was segmented determining the light intensity of the reference background from the narrow zone surrounding the nucleus using the cursor. Only a few nuclei in the centre of the image were measured because of the uneven illumination of the field of view. A rounded AOI with a stable size was used for selecting each nucleus separately throughout one measurement session. A total of 30-50 nuclei per slide were measured. If the parameter Integrated Optical Density (IOD) is selected with the Automatic Bright Objects option of the count/size command, then IOD is equal to Integrated Intensity of the nucleus to be measured.
Proposed method. Image processing protocol, selection of the parameters and data collection: Menu *Process?Color Channel?Extract*: Color Model - *RGB*, Generate channel – *B - OK*. Menu *Measure?Calibration*, select *Intensity*: click *New*, *Free Form*, *Options*: *Image*, define a 3x3 neighborhood template, use the cursor as crosshairs to determine the *Current Value* of light intensity of the background for calibrating the input value ? *OK,* select *Change* to calibrate 0 intensity for the y axis, *Calibration always positive - OK*. Menu *Edit?New AO ?*Select *Ellipse AOI* for measuring a nucleus *- OK*. Menu *Edit>AOI*: *Add* AOI with appropriate size, *Save*. Menu *Measur?Count /Size*, select *Automatic Bright Objects*, *Measure Objects, Accumulate Counts, Display objects.* Menu *Measur e?Data Collector ?Layout*: *Count Size* – selection: *IOD*; *?Data List?Collect Now* after *Count* in *Count /Size* menu; ? *Export?*Select *Excel (DDE)?Export Now*. The research was supported from DAAD and by the ESF grant No. 4989.

PS4-404-0243

Identification and characterization of differentially expressed genes following harvest of the *Lentinula edodes* fruiting body

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Lentinula edodes (shiitake mushroom) is a very popular edible, cultivated mushroom in Japan. There are post-storage problems with shiitake mushrooms, such as browning of the gills in the fruiting body or cell wall lysis, which can result in loss of fresh food quality and consequent loss of value. Lentinan is a cell wall component of beta-1, 3-linked-D-glucan with beta-1, 6 branches, which was isolated as an anti-tumor active-substance from *L. edodes*. Lentinan content decreases following harvest as a result of increased glucanase activity. To reveal physiological aspect of browning of fruiting body and cell wall lysis during post harvest preservation, we identified differentially expressed cDNAs during post-harvest preservation, by PCR-subtraction. Then we quantified transcriptional levels of the genes that were identified by PCR-subtraction.
Transcriptional profile of between fruiting bodies of day 0 (fresh) and day 3 following harvest were compared by PCR subtraction (PCR-SelectTM cDNA Subtraction kit; Clontech Japan). For the forward subtraction experiment, RNA from day 3 fruiting bodies was used as a "tester", and RNA from day 0 fruiting bodies as a "driver". The reverse subtraction experiment was performed oppositely. Transcriptional levels of the genes identified by PCR subtraction were quantified by real-time PCR. Full length cDNA of several genes identified by PCR-subtraction were cloned by 3' and 5' RACE PCR (SMART RACETM cDNA amplification kit, Clontech; GeneRacerTM kit, Invitrogen, respectively).
We identified laccase and tyrosinase encoding genes (*lcc4* and *tyr*, respectivery) in the forward subtraction. The *lcc4* was a novel laccase-encoding gene in *L. edodes*. Transcription of *lcc4* and *tyr* increased during post-harvest preservation, and these genes would be involved in browning of the fruiting body. We also identified several cell wall degradation-related enzyme-encoding genes, such as mixed-linked glucanase (*mlg1*), chitinases (*chi1, chi29*), chitin deacetylase (*chd1*). It is revealed that transcriptional levels of these genes increased after harvesting, by real-time PCR. Glucanase and chitinase activity increased following harvest as results of increased transcription of these cell wall degradation-related enzyme-encoding genes. Increase of transcriptional levels of these genes would cause cell wall lysis and lentinan degradation during post-harvest preservation. We identified many other genes, such as MAP kinase and sugar transporter-encoding genes, and sequenced many unidentified genes. In the reverse subtraction, we identified several proteases, peptidases, proteasome-related proteins, and heat shock proteins. These genes would be involved in physiological changes during fruiting body senescence following harvest.

PS4-405-0247

The role of primary germ tube for the morphogenesis of *Blumeria graminis*

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The conidia of *Blumeria graminis* f. sp. *hordei* (Bgh), following contact with the host surface, first form a short germ tube, called the primary germ tube (PGT), and then second, an elongating germ tube emerges. It differentiates into the appressorial germ tube (AGT), and then the AGT elongates and swells. It forms a hooked, appressorial lobe that penetrates the epidermal cell wall of the host. In a series of infections, the positive role of PGT for morphogenesis of the fungus is unclear except for the possibility reported by Carver and Ingerson that the growth of a long germ tube, with the potential to differentiate an appressorium, seems to be dependent on the perception of a suitable host surface through contact with the PGT. Therefore, the aim of the present studies is to further clarify the role of PGT for morphogenesis of the fungus.
The cuticle of barley coleoptile surface was removed with cellulose acetate and then conidia of *Blumeria graminis* were inoculated. The morphogenesis of the fungus such as PGT elongation or AGT emergence was observed at each time after inoculation.
When the conidia of Bgh were inoculated onto the coleoptile surface whose cuticle was removed with cellulose acetate, the emergence of the AGT was delayed.
This delay was related with the length of PGT, that is, on the cuticleless coleoptile surface, the PGT tended to continue elongating without stopping.
If there were gaps on the coleoptile surface such as a cell border on the more hydrophilic substratum like cuticle removed coleoptile surface, the PGT stopped elongating there and after that AGT seemed to emerge.
Then, we investigated that whether PGT elongation has stopped or not when AGT began to emerge. First, we recorded the length of PGT by taking the photograph of PGT when the AGT began to emerge. And then, we took the photograph of the PGT again after the AGT became APP and compared the length of PGTs of the first photograph with the second one. From these observations, it is clarified that PGT elongation has stopped when AGT began to emerge.
Stop of the PGT elongation is necessary for the trigger of AGT emergence.

PS4-406-0257

A homothallic mutant induced by UV irradiation in *Lentinula edodes*

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Shiitake, *Lentinula edodes* (Berk.) Pegler, is the major edible mushroom in Asia. The mating system of this fungus, leading to the formation of a dikaryon that can produce fruit bodies, is known to be bifactorial heterothallism (tetrapolality) controlled by two unlinked multialletic incompatibility factors, *A* and *B*. Mutants of *A* and *B* factors (*Amut* and *Bmut*) are useful for elucidating the structure and function of mating type genes. Particularly, an *Amut Bmut* double mutant is expected to have helpful properties that facilitate the recovery and analysis of developmental mutants. In previous studies, *B* mutants of this fungus had been obtained. However, no *Amut Bmut* double mutant has been isolated so far. To recover an *Amut Bmut* double mutant, basidiospores of the common *Bmut* dikaryon (*A1B1mut* x *A2B1mut*) were treated with UV irradiation. Of a total of 5000 monosporous isolates, a single basidiospore isolate was found to produce the hyphae bearing clamp connections without mating. The mutant could form fruit bodies on a sawdust medium, and all of the single basidiospore isolates from the mutant grew into colonies which were dikaryotic in appearance. These homothallic behaviors of the mutant were stably expressed for four generations. Microscopical observations of basidiospores treated with DAPI revealed that the mutant produced DAPI-negative, uninucleate or binucleate basidiospores. When basidiospores of the mutant were incubated on a thin film of malt extract agar medium, about half of basidiospores showed no sign of germination; the other half were able to germinate. During the germination and following hyphal elongation, a clamp connection was not observed at the first septum in two cells hypha, but clearly detected in a subsequent multicellular one which contained two nuclei in each cell. The clamp connections of the mutant were morphologically variable, viz., pseudo-, abnormal- and true-clamps. The germilings of the mutant were characterized by abnormal swelling and branching. To examine genetic relatedness among basidiospore isolates of the mutant, amplified fragment length polymorphism (AFLP) analysis was conducted. AFLP profiles among the basidiospore isolates were identical, indicating that the mutant produced isogenic basidiospores. These results suggest that the mutant recovered in this study is mutated in both *A* and *B* incompatibility factors.

PS4-407-0274

Growth parameters, morphological and genetic variability of the medicinal mushroom *Flammulina velutipes* (Curt. : Fr.) Sing.

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Recent advances in biotechnology have increased the interest in fungal organisms for developing new biotech-products and functional food additives. The *F. velutipes* contains different groups of bioactive compounds and enzymes with medicinal properties.

Mycelial micro-, macromorphological and genetic variability, as well as growth parameters, laccase activity test, pigmentation and telemorph formation of 31 collections of *Flammulina velutipes* have been investigated on malt-extract and potato-glucose agar media. The strains were isolated from fruiting bodies collected in different geographical regions and wood substrates.

Two morphotypes (A, B) and 3 subtypes (A1, A2, A-B) of mycelial colonies with different growth parameters were described in *F. velutipes* collections. Two forms with different intensity of agar pigmentation correlated with described morphotypes were observed. All strains of *F. velutipes*, except one, formed normal fruiting bodies on tested media. Correlations between morphotypes and growth characteristics with substrate nature and geographical origination of strains have been revealed. However, in order to evaluate species-specific media responses for described morphotypes further research with more strains is required. Mycelial macromorphology and growth parameters were more variable than described microstructures (form, shape of clamp-cells, crystals, oidia). Mycelia of tested *F. velutipes* strains were laccase positive. Significant correlation between the mycelial morphotypes and genetic variability of *F. velutipes* strains has not been revealed. Genes for morphological and cultural variability may be unlinked to genes for the nuclear ribosomal repeat.

Collections of putative *F. velutipes* were confirmed by nuclear rDNA-ITS sequencing to be *F. velutipes* with the exception of four collections identified as an unknown biotype, *F. elastica* and *F. rossica*. High genetic diversity was observed in Armenian collections of *F. velutipes* when compared to collections from Eurasia. At least 16 haplotypes were recovered in Armenian collections. This sequence diversity may be a consequence of the survival of an ancient genetic variation in the Caucasus and Armenia during glaciation period, while in Europe, the genetic variation was extirpated. A subset of Armenian genetic diversity of *F. velutipes* is found in Europe.

Revealed macro-, micromorphological and growth characteristics of mycelium will assist in further biotechnological cultivation of *F. velutipes*. High genetic variability of Armenian collections may be used for improvement of strains to obtain novel biotech-products and health-enhancing functional food additives from this valuable medicinal mushroom.

Research was supported in part by NATO (#980764), DAAD (#548.104401.174) and NSF PEET (DEB-9521526) grants.

PS4-408-0306

An investigation into the production of fungal extracellular mucilaginous material (ECMM) with relation to stress conditions

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Wood rotting basidiomycetes have been shown to produce copious amounts of extracellular mucilaginous materials (ECMM), however little specific evidence exists on the roles of ECMM in wood decay fungi. This is mostly non-quantitative and relates to general concepts such as attachment to the substrate and entrapment of decay agents. Two basidiomycete fungi, the white rot fungus *Coriolus versicolor* and the brown rot fungus *Gloeophyllum trabeum*, have been used as model organisms to investigate the role of ECMM in their responses to a number of physiological factors relevant to wood decay. It was postulated that ECMM would have a protective role for the fungal mycelium serving to isolate sensitive hyphae, and in particular hyphal tips, from adverse environmental conditions.
Under conditions of stress induced by physiological conditions and by the presence of toxic chemicals, the amount of ECMM produced as a proportion of the total biomass by the test organism increased. This was always associated with a decrease in the overall total amount of biomass produced. A shift was observed in the carbohydrate composition and other components, such as the amount of protein in the ECMM, under the range of conditions tested.
Under stress conditions, the mycelia of both fungi became more highly branched. This response was interpreted as being the underlying mechanism delivering the increased proportion of ECMM observed under the various conditions, since the hyphal tips are known to be the active sites for ECMM production.
In vitro experiments were also conducted on the ability of copper sulphate to diffuse through ECMM. These demonstrated that ECMM significantly reduced the diffusion rate of copper sulphate and provided support for the hypothesis that ECMM can protect hyphae from toxins.
The results of these studies have demonstrated that two wood decay basidiomycete fungi produce increased amounts of ECMM under stress conditions and that this is associated with a change to a more highly branched colony morphology. Qualitative changes also occurred in ECMM under a range of growth conditions, which indicated that ECMM is a very dynamic material, which can adapt according to the conditions experienced by the fungi. Such compositional variations may reflect in the roles fulfilled by ECMM during fungal growth. The results are discussed with regard to the possible physiological roles of ECMM during wood decay and the potential of ECMM to bind toxic or potentially toxic substances, hence reducing their accumulation in fungal hyphae.

PS4-409-0307

Mode(s) of action of chitosan: 1. The effect on fungal cell membranes

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Chitosan is an abundantly occurring biopolymer obtained from the N-deacetylation of chitin. In recent years, chitosan has been the focus of research not only because of its adjuvant and elicitation properties, but also because of its ability to control fungal growth. Special interest has been shown in the use of chitosan for the control of wood degrading fungi. However, little specific evidence is available to elucidate the modes of action of chitosan and the mechanisms through which its antifungal activity is mediated.
The present study postulated that chitosan toxicity exerts an effect on membrane permeability and on the architecture of the mycelium. The two wood degrading fungi *Sphaeropsis sapinea* and *Trichoderma harzianum* were used as a model to investigate the effect of chitosan on membranes and their functionality and also on hyphal growth.
The focus of our work was to investigate common stress responses at cellular level, such as the production of reactive oxygen species (ROS) and intracellular K+ leakage. Increased production of hydrogen peroxide and superoxide was observed especially during the initial stages of growth. Treatment with catalase caused an increase in radial growth of both species, even in the presence of chitosan, suggesting that the onset of oxidative stress might be partly responsible for the growth reduction observed due to chitosan treatment. Increasing concentrations of chitosan also caused an increase in K+ from fungal cell. Taken together these observations suggest that the plasma membrane could be the primary target of chitosan action.
Examination of the hyphae using light and electron microscopy techniques showed that chitosan induced alterations in hyphal morphology and ultrastructure. Increasing concentrations of chitosan induced excessive branching in fungal hyphae. Electron microscopy also revealed morphological and ultrastructural changes in the membranes.
The implications of these results are discussed in relation to the potential mechanisms mediating the efficacy of chitosan as an antifungal agent. The benefits to the wood preservation industry that the use of chitosan can bring as a "biopreservative" are also discussed.

PS4-410-0308

Mode(s) of action of chitosan: 2. The effect on fungal cell wall deposition

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Chitosan is a biopolymer obtained from the N-deacetylation of chitin. The efficacy of chitosan as an adjuvant and a plant elicitor are well documented and interesting results have been obtained in the use of chitosan for controlling fungal growth. However, little specific evidence is available to elucidate the mechanisms through which its activity is mediated.
In the present study, two wood-inhabiting species, *Sphaeropsis sapinea* and *Trichoderma harzianum*, have been used as a model to investigate the effect of chitosan on the cell wall deposition. We postulated that increasing concentrations of chitosan would cause an increase in chitin deposition, which would reflect changes occurring at morphological and ultrastructural level within the cell wall.
The study employed three different techniques in order to quantify chitin in the fungal mycelium. A colorimetric method for the detection of D-glucosamine was compared with two methods using GC-MS *pyro*GC-MS. All methods provided evidence of an increase in the chitin content in the mycelium in the presence of increasing concentrations of chitosan in the growth medium, suggesting that chitosan treatment enhanced deposition of cell wall. The effect of the presence of chitosan on the reliability of the three methods was also evaluated.
Transmission electron microscopy was used to determine whether such increase in the amount of chitin was due to increased cell wall thickness or to a more compact cell wall architecture.
The implications of these results are discussed with a view to analyzing the mechanisms associated with growth inhibitory effects of chitosan on fungal hyphae. The benefits related to the use of chitosan as an environmentally benign substitute for traditional hazardous chemical wood preservatives are also discussed.

PS4-411-0312

An F-actin depleted zone is present at the hyphal tip of invasive hyphae of the ascomycete *Neurospora crassa*

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F-actin is thought to be a key player in tip growth in both fungi and oomycete hyphae. In these evolutionarily distant, yet morphologically similar groups of organisms it has been hypothesized to play a number of roles in morphogenesis including the control of tip yielding and vesicle delivery to the tip. F-actin is typically seen at a high concentration at the tip, although there have also been some indications of F-actin depleted areas in the tip of some species of fungi. We have recently reported that, in the oomycetes, this F-actin depleted zone is associated with invasive hyphae. As the hyphal growth form is suspected to have arisen by convergent evolution in oomycetes and fungi this raises the question of whether an F-actin depleted zone is also a feature of fungal hyphae. In view of the above we have carried out an investigation of the distribution of F-actin, the F-actin severing protein cofilin and vesicles in invasive and non-invasive hyphae of the ascomycete *Neurospora crassa*.
Both non-invasive and invasive hyphae were grown on scratched cellophane overlaying 2% agar containing Vogel's minimal medium with 1.5% (w/v) sucrose. The invasive hyphae were overlaid with 2% low melting point agar. After growth recovery hyphae were chemically fixed with 4% paraformaldehyde and 0.5% methylglyoxal and stained with an anti-actin or anti-cofilin antibody. Live hyphae were also exposed to the membrane sensitive dye FM-4-64 for visualisation of vesicles and the Spitzenkörper. Stained hyphae were observed using epifluorescent and confocal microscopes.
We found that 86% of non-invasive hyphae had a tip high concentration of F-actin, this compares to only 9% of invasive hyphae. The remaining 91% of the invasive hyphae had no obvious tip high concentration of F-actin staining; instead they had an F-actin depleted zone in this region. The membrane stain FM4-64 revealed a slightly larger accumulation of vesicles at the tips of invasive hyphae relative to non-invasive hyphae, although this difference is unlikely to be sufficient to account for the exclusion of F-actin from the tip. An anti-cofilin antibody localised to the F-actin depleted zone of invasive hyphae.
We suggest that the F-actin depleted zone may play a role in the regulation of tip yielding to turgor pressure, thus increasing the protrusive force that might be necessary for invasive growth. The rearrangement of the cytoskeleton to create this depleted zone may come about through the action of the F-actin severing protein cofilin.

PS4-412-0342

Respiration properties of Pythium species from cool-temperate forest soil

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The final goal of this research aims to investigate a prospect of *Pythium* species to use as an indicator for an estimation of soil microbe respiration. In this study, we examined respiration properties of *Pythium* species from cool-temperate forest soil.

Pythium spinosum, *P. sylvaticum*, and *Pythium* group HS isolates collected from cool-temperate forest soil in Japan were used. The isolates were cultured on Schmitthenner's agar medium and incubated at 5, 10, 15, 20 and 25°C. The concentration of CO2 in culture container was measured at 0, 8, 16 and 24 h after incubation. To examine the relationship between mycelial growth and respiration, the isolates were cultured in Schmitthenner's broth medium at 25°C. The concentration of CO2 was measured 48, 72 and 96 h after incubation. Dry weight of mycelium was measured as mycelial growth. Respiration rate was calculated as released CO2 per g dry weight of mycelia.

Results and Discussion

The respiration activity of *Pythium* species changed in response to temperature. *P. spinosum* showed the highest respiration activity at more than 20°C. On the other hand, the HS group tended to release more volume of CO2 at 15°C than *P. sylvaticum* and *P. spinosum*. At less than 10°C, there was no difference in the volume of released CO2 among the investigated species, in which the respiration activities were low.

The volume of released CO2 was positively correlated with the mycelial weight, regardless of species. Although *P. spinosum* tended to release more volume of CO2 than *P. sylvaticum* and the HS group, the rate of released CO2 per mycelial weight was greatest in the HS group.

The results suggested that the response to temperature differed among *Pythium* species and that the volume of released CO2 could be estimated based on the inoculum density. The HS group that was predominant in cool-temperate forest soil might have adapted to cool environment.

PS4-413-0391

A strictly aquatic fungus can biotransform 1-naphthol

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Aquatic hyphomycetes are specifically adapted to aquatic environments, which initiate the decomposition of organic matter arising from the riparian vegetation in streams and lakes. Knowledge about their potential to degrade xenobiotic compounds is limited to a few examples [1, 2].

Besides bacteria, environmentally ubiquitous filamentous fungi and yeasts were implicated to degrade aromatic hydrocarbons in natural ecosystems.

1-Naphthol is a toxic microbial degradation metabolite of the widely used broad-spectrum insecticide carbaryl (1-naphthyl-N-methyl carbamate) and was found to contaminate surface waters and sediments at carbamate pesticide production sites.

The strictly aquatic fungus *Heliscus lugdunensis*, isolated from a high polluted habitat [2], metabolized approximately 74% of 1-naphthol within 5 days. The identification and quantification of degradation products using GC-MS, LC-MS, and HPLC revealed that approximately 12% of the parent compound was converted into 1naphthylsulfate, 3% was transformed into 1-methoxy-naphthalene, and less than 1% was converted into 1,4naphthoquinone.

The work was done to broaden the knowledge about the potential of exclusively aquatic fungi to affect the environmental fate of pollutants of xenobiotic origin in freshwater environments and the biochemical reactions involved.

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PS4-414-0400

Carbon and Nitrogen Dynamics of Mycena epipterygia Decomposing Pine Needles

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Anthropogenic nitrogen inputs to many forest ecosystems have increased but their effects on litter decomposition are not fully understood. It has for long been a dogma that decomposer systems are C-limited, since additions of labile carbohydrates increases respiration. Many studies suggest that litter degrading microorganisms are often limited by low N availability. However, additions of N have often been observed to have no or negative effects on decomposition, particularly the decomposition rate of more recalcitrant material seen in a longer time perspective. Underlying these contradicting results are methodological problems associated with field experiments with complex microbial communities and food webs. Additions of labile C or N may lead to drastic changes in the microbial community. The relationship between microbial growth and decomposition rates is also difficult to monitor in field experiments. Detailed laboratory studies of decomposition under controlled conditions and with known decomposer organisms may potentially shed light over the contradicting observations made from field conditions.
The aim of this study is to evaluate the potential of the litter decomposing fungus *Mycena epipterygia* to alter its C-use efficiency (C biomass/C assimilated) in response to increased N and C availability. The fungus was grown axenically on Scots pine needles and the effects of added glucose or ammonium on C-use efficiency were studied by measuring the respiration and fungal biomass production over a 38 day period. Respiration was measured using an Infra Red Gas Analyser (IRGA) and chitin analysis was used to estimate fungal biomass production.
Preliminary results indicate that N addition immediately increases respiration from pine needles in early stages of decomposition. However, addition of glucose also increased respiration, but after a one week lag phase. The results of the calculated C-use efficiency will be discussed in relation to the decomposition responses observed after N addition.

PS4-415-0404

Selenium induces manganese peroxidase production by the white-rot fungus LSK-27

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Most white-rot fungi species secrete lignin-modifying enzymes such as manganese peroxidase (MnP), laccase, and lignin peroxidase during their secondary metabolism which is generally triggered by nitrogen and/or carbon deprivation. Various specific mechanisms for induction of enzyme production were previously reported in detail. However, to our knowledge, the effects of selenium as an inducer for ligninolytic enzyme production in white-rot fungi has never been studied. We demonstrated that selenium either induced or repressed MnP production in white-rot fungus LSK-27, in a dose-dependent manner. While the higher concentrations of selenium repressed the MnP production, lower selenium concentrations stimulated it by about 3 fold. Interestingly, smaller selenium concentrations caused a decrease in the extracellular glutathione levels. These preliminary results indicate that selenium might protect the organism from the oxidative stress, in a manner similar to that in higher eukaryotes.

PS4-416-0419

The H+-ATPase LmPMA1 is involved in pathogenicity of Leptosphaeria maculans on oilseed rape

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Leptosphaeria maculans, the causal agent of stem canker, is the major pathogen of oilseed rape all over the world. In order to decipher the fungus infection strategies, and in parallel to the current genome initiative (see Balesdent et al. communication), a collection of 3000 *Agrobacterium*-mediated transformants has recently been generated and characterized in our laboratory. Here, we describe the phenotypic and functional characterization of one non-pathogenic mutant, m210. M210 is morphologically similar to the wild type isolate *in vitro* and shows no growth or sporulation defect. It induces a typical hypersensibility reaction on susceptible oilseed rape leaves and is totally inefficient to colonize the stem. Formal genetic studies performed on a progeny of 54 isolates showed an exact co-segregation between the m210 phenotype and the selection marker, thus confirming the efficient tagging of the mutant. T-DNA border sequencing allowed us to localize its insertion within the promoter of one gene, 274 bp upstream the start codon. The insertion results in the deletion of 7 bp of the promoter region. This gene is homologous to the highly conserved fungal gene *PMA1*, which encodes the predominant and essential plasma membrane H+-ATPase. The basic function of this protein in fungal cells is to create an electrochemical proton gradient, which drives the uptake of nutrients by secondary active transport systems and regulates the intracellular pH. The *Leptosphaeria maculans* H+-ATPase possesses all the characteristics common to other fungal H+-ATPases (conserved catalytic and transmembrane domains). Quantitative RT-PCR analyses showed that *LmPMA1* is expressed at a high level and in a constitutive way *in vitro* (germinating conidia, mycelia) and *in planta*. In m210, the T-DNA insertion induced a 50% reduced expression of *LmPMA1* in *in vitro* growing mycelium but not in germinating conidia, compared to the wild type. We suggest that the separation of transcriptional regulation boxes from the gene start by the T-DNA insertion led to a deregulation of the expression in m210. In fungi, the expression of the H+-ATPase is under the control of environmental pH and morphogenic development. We are actually investigating the influence of these two factors on *LmPMA1* expression, both in the wild type and mutant strains.

PS4-417-0435

Characterization of Cdk-related kinases from Blastocladiella emersonii during its life cycle

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Cyclin-dependent kinases are key enzymes responsible for the control of the cell cycle progression. While in fungi, only one Cdk, named Cdk1, has been described as directly involved in the control of cell cycle transitions, in animals at least two Cdks have been indicated and members from two distinct groups could be involved in cell cycle control in plants. One characteristic of chytridiomycetes is their growth as a coenocyte without citokynesis until their entry in the sporulation phase, or briefly thereafter. Although cell cycle control mechanisms seem to be conserved in eukaryotes, mechanisms and control points of the transition from a nucleated cell to a multinucleated cell that is finally divided into cellular compartments remain obscure. As a first step towards understanding the role of the cell cycle in the life cycle of the chytridiomycete *B. emersonii*, we report the identification of two putative Cdk or Cdk-related kinase (Crk) cDNAs and their characterization by sequence analysis and mRNA levels. The deduced protein sequences from the isolated cDNAs present the conserved motifs of Cdks, but the characteristic PSTAIRE motif in their cyclin-binding domain is divergent. Both Crk genes showed pre-translational regulation, but with different mRNA profiles along the fungus life cycle. Post-translational regulation is a characteristic of Cdks, with one plant group described as transcriptionally regulated. Cdk associated activity was also investigated in immunoprecipitates obtained with polyclonal antiserum produced against one of the Crks and in co-precipitates with p13Suc1, a high-affinity Cdk regulatory subunit that associates with active enzyme complex. Both patterns of histone H1 phosphorylation showed kinase activity throughout the life cycle with a reduction at the end of sporulation, which accompanied the mRNA profile. Moreover, both activities were inhibited with purvalanol A, a selective and potent Cdk inhibitor. This is the first report of putative Cdks of a chytridiomycete, which appear to be different in sequence and expression from those described for other fungi.

PS4-418-0449

Genes involved in sclerotial differentiation in Sclerotinia sclerotiorum.

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Sclerotinia sclerotiorum is a ubiquitous, necrotrophic, ascomycetous fungus that infects over 400 plant species including many economically important crop species. During its lifecycle this highly successful pathogen can form hardy resting structures called sclerotia that allow it to over-winter in the soil. The questions we are interested in answering are: What molecular pathways co-ordinate sclerotium formation? And are such mechanisms homologous to conidiogenesis regulation pathways in unrelated filamentous fungi? A starting point for this work has been, firstly, to identify genes differentially expressed during sclerotial initiation and secondly, to find *Sclerotinia* genes that are homologous to genes known to regulate conidiogenesis in other fungi. We are currently using expression knock-down to test the effect of these genes on sclerotial morphogenesis.

AFLP differential display was used to compare genes expressed during sclerotium formation in a wild type strain with gene expression in a non-sclerotial UV mutant. Candidate genes homologous to known conidiation regulatory genes were identified from the *Sclerotinia sclerotiorum* genome sequence (http://www.broad.mit.edu/annotation/fungi/sclerotinia_sclerotiorum/). Northern blots were used for analysis of expression during sclerotial initiation. *Agrobacterium*-mediated transformation and RNAi are being used to specifically disrupt expression of the candidate genes.

AFLP and Northern analysis identified several genes that are specifically expressed during sclerotial morphogenesis. These genes include genes putatively involved in sugar transport and secondary metabolism. One of the genes identified by AFLP (800AT) has sequence similarity to the *fluffy* (*fl*) gene which is the major regulator of conidiation in *Neurospora crassa*. 800AT amino acid sequence was 28% identical to *fl* (over 50% of the protein). There was 62% identity between the *fl* DNA binding domain and the corresponding domain on 800AT including residues important for DNA binding. We are currently using RNAi-induced silencing to examine the effect of loss of expression of the putative *fl* homologue on sclerotial morphogenesis.

The development of a transformation system based on *Agrobacterium tumefaciens* has provided the ideal tool for creation of gene knock-outs and knock-downs in *S. sclerotiorum*. We have identified a candidate gene with sequence similarity to the *N. crassa fluffy* gene. This novel gene contains the DNA binding domain, basic region and middle homology region typical of a GAL4-type transcription factor and is differentially expressed during sclerotial formation. As some types of sclerotia are considered to be evolutionarily derived from conidiogenous tissue, we anticipate considerable homology between regulation of conidiogenesis and sclerotia formation at a molecular level.

PS4-419-0461

Pleiomorphic vacuoles in Aspergillus oryzae and their possible involvement in nutrient recycling

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Vacuoles in filamentous fungi are highly pleiomorphic and some of them, e.g. tubular vacuoles are implicated in intra- and intercellular transport. We established *Aspergillus oryzae* strains that express the functional fusion protein of enhanced green fluorescent protein with AoVam3p (EGFP-AoVam3p), a putative vacuolar t-SNARE, and carried out microscopic observations. FM4-64 and CMAC staining confirmed that EGFP-AoVam3p localized on the membrane of the pleiomorphic vacuolar networks, including large spherical vacuoles, tubular vacuoles, and putative late endosomes/prevacuolar compartments. The vacuoles changed their shape and size over time, as revealed by time-lapse imaging of EGFP-AoVam3p with a confocal microscope. In addition, EGFP-AoVam3p-expressing strains led to the discovery of several new aspects of fungal vacuoles, which have not been discovered so far with conventional staining methods, during different developmental stages. In old hyphae, EGFP fluorescence was present in the entire lumen of large vacuoles, which occupied most of the cell, indicating that degradation of cytosolic materials had occurred in such hyphae via an autophagic process. Since these vacuoles were often interconnected with tubular vacuoles, the cellular components of old hyphae may be recycled to more active regions of the mycelium via the tubular vacuoles. In hyphae that were not in contact with nutrients, such as aerial hyphae and hyphae that grew on a glass surface, vacuoles were composed of small punctate structures and tubular elements that often formed reticular-like networks. This observation suggests that the tubular vacuoles may be involved in nutrient transport to these hyphae.

PS4-420-0476

The Coprinus cinereus eln6 gene involved in stipe elongation during fruit body maturation encodes a putative glycosyl transferase.

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The stipe cells of the mushroom *Coprinus cinereus* exhibit rapid elongation during fruit body development, providing an excellent opportunity to study molecular mechanisms underlying fungal cell morphogenesis.
We isolated an elongationless mutant that is defective in elongation of the fruit-body stipe of *C. cinereus*. Microscopic observations revealed that the stipe cells in the mutant not only fail to elongate normally, but also exhibit crooked form even before elongation. Linkage analysis using RAPD markers mapped the gene responsible for the elongationless phenotype, *eln6*, on chromosome XIII. We constructed a BAC library of the Okayama-7 strain using a vector carrying the *C. cinereus trp1* gene as a selectable marker, and then assigned BAC clones to specific regions of the published genome sequences by fingerprinting, the BACFinder program and BAC end-sequencing. We transformed an *eln6-1* mutant strain carrying the *trp1* auxotrphic marker with BACs assigned onto chromosome XIII, and identified a BAC clone that complements the *eln6-1* mutation.
We identified the *eln6* gene in the BAC clone by testing the *eln6* activity of PCR amplified fragments from the BAC clone with the *trp1* marker gene. The *eln6* gene is predicted to encode a putative membrane protein of 881 amino acids with a glycosyl transferase domain. The *eln6-1* mutant gene has substitution of 2 base pairs which truncates the C-terminal region including the glycosyl transferase domain, suggesting a loss of function of the Eln6 protein. Eln6 exhibits a strong similarity to Eln3, which has been identified by analysis of another elongationless mutant of *C. cinereus* and shown to be a putative membrane protein with a general glycosyltransferase domain. These results suggest that Eln6, together with Eln3, is involved in production of a cell wall component(s) that is essential for the stipe cells to elongate.

PS4-421-0491

Isolation and in vitro cultivation of the fastidious insect pathogenic fungus Cordyceps unilateralis

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Cordyceps unilateralis is an ant pathogenic fungus with a world-wide distribution which is common in Thai forests. Of 120+ species of *Cordyceps* recorded from Thailand this has proved the most difficult to get in culture. A method for the isolation and *in vitro* growth of this fungus was, therefore, developed.
Ascospores from infected ant cadavers were used as starting materials and their secondary conidia were allowed to germinate first on Potato Dextrose Agar. These secondary conidia were induced to develop further growth by transferring them to Grace's Insect Tissue Culture Medium. Then, the formation of mycelia was stimulated by using Potato Dextrose Broth. As a result, the *in vivo* life cycle of *C. unilateralis* from the steps of secondary spore germination, blastospore formation and mycelium growth was successfully completed *in vitro*.
These three steps of development facilitated the efficient isolation and cultivation of *C. unilateralis* in the laboratory allowing us to secure 150+ isolates of *Cordyceps unilateralis*. In addition, the effect of nutritional factors on blastospore and mycelial growth was investigated. Glucose was found to be the most important factor for blastospore formation while yeast extract could be used as a nitrogen source. Addition of salt solutions to the basic Grace's medium was found to be essential for blastospore formation.

PS4-422-0497

The search for polyketide synthase genes producing beta-orsellinic acid and methylphloroacetophenone as precursors for beta-orcinol depsidones and usnic acids in the lichen Chondropsis semiviridis

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Unique aromatic polyketide compounds produced solely by lichens have shown diverse biological activities. The aim of this study is to identify the polyketide synthase (PKS) genes responsible for the production of the hypothetical precursor of beta-orcinol depsides and depsidones, and usnic acids in lichens. All three groups of compounds are predicted to arise from a common precursor (a C4-methylated C8 polyketide chain), which is later cyclised into beta-orsellinic acid or methylphloroacetophenone. It is likely that these PKS gene products are of the non-reducing type and possess a methyltransferase (MT) domain. Using a phylogenetic approach combining PCR and Southern hybridisation, we attempted to identify the potential genes for the precursors in *Chondropsis semiveridis* (F.Muell. ex Nyl.), where beta-orcinol depsidones (i.e. succinoprotocetraric acid and fumarprotocetraric acid) and usnic acid is detected in the thallus by HPLC. Degenerate primers biased to the non-reducing (NR) clade III PKSs based on Kroken et al. (2003) were designed to amplify the ketosynthase (KS) domains. Phylogenetic analysis indicated that *C. semiviridis* has two such KS domains (KSc3 and KSc4), both showing >70% amino acid identities to various non-reducing type PKS in that clade. Southern hybridisation of the genomic DNA with the KS domains showed two bands for KSc3 and a single band for KSc4. The region corresponding to a 4 kb BamHI-HindIII band that hybridized with KSc4 was excised from gels and subcloned. Sequencing of positive clone, p52KS, revealed a 2.3 kb 5' fragment of a PKS gene and a partial fragment of a putative esterase gene located 1.5kb upstream with an opposite direction of transcription. The downstream sequence of the KS domain was obtained by a domain-hopping strategy. Two 400 bp fragments amplified using degenerate primers targeting the MT domains of NR PKSs were cloned and sequenced. The reverse primer was designed from a MT fragment showing similar BLASTx matches and was used to obtain a 4.5 kb PCR product between the KS and MT domain. BLASTx search of the combined 6.3 kb partial PKS sequence indicated that citrinin PKS from *Monascus purpureus* (pksCT) was the closest PKS homolog and that both shared the same domain orientation. The relative location of the putative esterase and PKS genes suggests that they both form part of a beta-orcinol depside or depsidone gene cluster, as they fit the enzymatic steps required. Additional experimental data, including heterologous expression of the genes, is needed to support the prediction.

PS4-423-0498

Identification of mating genes in the Aspergillus oryzae genome: studies towards understanding their role in its life cycle

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Aspergillus species exhibit both asexual and sexual modes of propagation with homothallic or heterothallic patterns of breeding. While in *A. nidulans*, a homothallic species, sexuality is well known and sex in *A. fumigatus* is beginning to be understood, not much is known on the closely related species, *A. oryzae*, which is considered to propagate "asexually". In contrast to *A. nidulans* with both *MAT1-1* and *MAT1-2* loci, typical of its homothallic nature, the genome of *A. oryzae* RIB40 strain revealed the existence of only *MAT1-1* gene encoding a protein with alpha-box motif, prompting a consideration on its sexual identity. Interestingly, PCR analysis in several industrial strains of *A. oryzae* using *MAT1-2* gene specific primers revealed the presence of *MAT1-2* gene encoding a protein with HMG-box motif, confirming the existence of opposite mating type strains in *A. oryzae*. Moreover, the presence of genes encoding the alpha-pheromone (*AoppgA*) and its receptor (*AogprA*), which are indispensable for sexual response, in addition to genes encoding the proteases (Kex1, Kex2 and Ste13) required for processing of alpha-pheromone implicated probable sexual pathway in *A. oryzae*. While RT-PCR analysis confirmed the expression of *AoppgA* and *AogprA* genes, their expression was upregulated upon carbon source depletion suggesting that recognition of alpha-pheromone by its receptor might play an important role during carbon starvation. The AoPpgA protein with 103 aa contained a putative secretory signal sequence, and two repeats of ~10 amino acids each followed by Kex2-processing site as in other fungal alpha-pheromones. Processing and secretion of AoPpgA in *A. oryzae* was confirmed by the expression *AoppgA-egfp* fusion construct under the control of *amyB* promoter. EGFP fluorescence was detected at the septa and in the vacuoles, which is usually observed when a secretory protein fused with EGFP is expressed in *A. oryzae*. Coomassie staining and Western blotting using GFP-antibody suggested the secretion of the processed forms of AoPpgA into growth medium. The *AogprA* encoded a protein belonging to the G protein-coupled receptor family and the AoGprA-EGFP fusion localized at the plasma membranes. To further characterize the function of alpha-pheromone and its receptor, the *AoppgA* and *AogprA* disruptants were generated. Phenotypic analysis of the ¢*AoppgA* and ¢*AogprA* strains showed reduced conidiation in comparison to the wild type. The results obtained thus far suggest that the *AoppgA* and *AogprA* genes may have other functions in *A. oryzae*.

PS4-424-0532

Isolation and screening of biologically active metabolites of selected marine fungi from Malaysia

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Many marine fungi have been screened for their biologically active secondary metabolites and vast numbers of new biologically active substances have been discovered. This study was conducted to isolate marine fungi on decaying wood of mangrove trees, *Nypa fruiticans* and driftwood from coastal areas and to screen marine fungi producing antimicrobial activities against bacteria and yeasts in Malaysia. Studies on diversity of marine fungi were carried out at Morib, Kuala Selangor and Langkawi Island from 2003 to 2004. One hundred fifty two marine fungi were successfully isolated and cultured at the Herbarium, University of Malaya and these isolations were screened for antimicrobial activities using plug assay. In the plug assay, three yeasts and four bacteria namely, *Candida albicans, Saccharomyces cerevisiae, Schizosaccharomyces pombe*, *Bacillus subtilis, Escherichia coli, Klebsiella aerogenes and Staphylococcus aureus* were used as test organisms. In this study only 66 marine fungi showed positive activity against bacteria and yeasts. The diameter of inhibition zones range from 9-20 mm for the plug assay. Results indicated that 64 marine fungi exhibited antibacterial activity against *B. subtilis,* 63 marine fungi agains*t S. aureus,* 11 species against *K. aerogenes* and 5 species against *E. coli.* Whereas, the number of marine fungi exhibiting antifungal activity against *C. albicans, S. cerevisiae,* and *S. pombe* are 6, 12 and 14, respectively. Only seven marine fungi inhibited the growth of both bacteria and yeasts. Five species of marine fungi selected for further screening were *Caryosporella rhizophorae, Fasciatispora nypae, Melaspilea mangrovei, Leptosphaeria* sp. and Asco sp. 19(NF). A disc diffusion assay using ethyl acetate extracts from 20 days old cultures was also carried out to confirm the plug assay results. The diameter of inhibition zones range from 9-16mm for disc diffusion assay. Time-course studies were also carried out to estimate the rate of secondary production. Two different conditions were employed: static/stationary and shake at 250 rpm. In general, activities against yeasts were more prominent in stationary incubation and most of the extract cultured showed activity after 15 days. Whereas in shake incubation, *C. rhizophorae* showed good activity against bacteria after 10 days. The result confirms our earlier observations from the plug assay. Cytotoxicity studies and chemical characterization of these extracts are planned.

PS4-425-0542

Zoosporangium development and zoospore release of Halophytophthora kandeliae

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Halophytophthora species were mainly isolated from intertidal fallen leaves in subtropical and tropical mangrove forest. Currently fifteen species and two varieties are known, and species delimitation is based mainly on the morphology of zoosporangium, and on the characters of vesicle, plug and operculum. However, the great diversity in the morphology of zoosporangia and in the mode of zoospore release suggested this genus could be polyphyletic. To clarify this taxonomic problem, detailed studies of zoosporangium development in different species are necessary, which may provide useful information in taxonomic composition of this genus. A species, *Halophytophthora kandeliae,* distinguished from other *Halophytophthora* species primarily by its regularly obovate sporangia and by forming spherical, persistent vesicle, was examined for the development of zoosporangia and the process of the zoospore release by using LM, SEM and TEM. Our results showed the presence of operculum which opened through the predetermined line at the apex region of zoosporangium wall. Spherical vesicle was formed from crescent-shaped translucent matrix beneath the apex of zoosporangium. Cytoplasm cleavage and then zoospores developing occurred after part of zoosporangial cytoplasm ejected into spherical vesicle. The wall (or membrane?) of vesicle became gradually digested during zoospore developing, and then ruptured to release mature zoospore. The results revealed one of the several modes of zoospore release in *Halophytophthora* species, which are unique only in *H. kandeliae*.

PS4-426-0546

The relative importance of different groups saprophytic and mycorrhizal fungi revealed by a flux model of dead plant matter and belowground assimilate allocation in Norway spruce forests.

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Fluxes over time rather than the amount at a given point of dead plant material (DPM) or allocation of assimilate belowground in forest ecosystems are important, not only for terrestrial carbon exchange but also for forest biodiversity and for fungal diversity. The majority of forest organisms are saprophytic and integrated in food-webs emanating from either DPM or mycorrhizal systems. Fungi carry out the predominant part of decomposition in boreal forests. In these forests, threatened species are largely confined to virgin forest conditions or to coarse woody fractions of DPM. Yet, little attention has been paid to the relative importance for forest organisms of coarse and fine dead wood in relation to other DPM such as needles, leaves and fine-roots from the trees as well as DPM from the field- and bottom-layer. The relative activity of ericod- and ectomycorrhiza in boreal forests is similarly incompletely known. This type of analyses need to be temporal as the magnitude of fluxes of DPM and assimilate allocated belowground vary during forest succession.

Here we present a model of the relative contribution of different fractions of above and belowground DPM from trees, field- and bottom-layer and the amount of assimilate allocated belowground from trees and field-layer. The analysis compare managed and virgin Norway spruce forests during a forest generation. We have acquired and compiled data of above and belowground growth and production of DPM of trees, field-and bottom-layer and modelled the inflow of various fractions of DPM as well as the root-production as a measure of belowground assimilate allocation during a forest generation. In order to display the importance of different DPM fractions over time for saprotrophic organisms, we calculated the heterotrophic respiration rather than showing the inflow and standing mass of DPM.

Using this model we show that heterotrophic respiration originating from dead fine-roots and needles largely exceed that from wood. We also show the potential amount of assimilate allocated to the communities of ericaceous and ectomycorrhizal fungi, respectively, over time and at conditions with different soil fertility.

PS4-427-0563

The litter-decomposing fungus Mycena epipterygia produces a novel hybrid enzyme of lignin and phenol-oxidizing peroxidases

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The genus *Mycena* represents a diverse group of about 500 species of agaric mushrooms, which worldwide colonize soil-litter and plant debris in forests. An agar-plate screening, including 19 *Mycena* spp. (30 different strains) from Middle Europe and Scandinavia, was performed to select strains producing extracellular oxidoreductases such as laccase, manganese peroxidase and/or other peroxidases. Only two strains did not show any oxidative activity and 24 strains were able oxidize the indicator substrate ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate). All ABTS oxidizing strains secreted laccase but significant peroxidase activities were only found in three strains of *Mycena epipterygia*. This litter-decomposing fungus, colloquially called Yellowleg Bonnet, is a widespread and common mushroom, which lives on dead leaves and needles. It prefers to fruit on or under conifers and produces fruiting bodies, which are small and slimy-capped typical agarics. The production of peroxidases by *M. epipterygia* was studied in surface and agitated cultures using a complex N-rich medium based on soy bean meal. Highest peroxidase levels were detected in stirred-tank bioreactors (10 liter), reaching up to 800 Units l-1 8 days after inoculation. In classic N-depleted and other synthetic media mostly used to obtain ligninolytic activities (Kirk, Czapek Dox), the fungus secreted only very low peroxidase activities. After several concentration and purification steps, a classic manganese peroxidase and a novel heme peroxidase were isolated from the culture liquid. The latter enzyme oxidized both phenolic substrates such as 2,6-dimethoxyphenol as well as the non-phenolic aromatic compounds as veratryl alcohol (VA) and ?-O-4 lignin model dimers. Thus this *Mycena* peroxidase is the first VA oxidizing peroxidase of a litter-decomposing fungus and it combines properties of classic phenol-oxidizing peroxidases (e.g. horseradish peroxidase, *Coprinus cinereus* peroxidase) and lignin peroxidase from ligninolytic white-rot fungi. On the other hand, the enzyme is not capable of oxidizing Mn(II) ions as fungal manganese or versatile peroxidases do, and hence it may represent a novel hybrid form of ligninolytic biocatalysts. Biochemical characterization of the enzyme is currently under investigation. Preliminary tests indicate that *Mycena* peroxidase is a comparatively large (~ 52 kDa) and heavily glycosylated heme protein.

PS4-428-0568

Proteolytic activity of several Coprinoid mushrooms

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Many fungi including basidiomycetes mushrooms produce extra-cellular proteolytic enzymes of great commercial importance. Several of these fungi can be used in food and medicinal industry to obtain milk-coagulating, proteolytic, thrombolytic and fibrinolytic biotech-products. Enzymatic complexes with caseinolytic, milk-coagulating, fibrinolytic and thrombolytic activities produced by Coprinoid mushrooms have been reported before by Denisova (1990). Physiological and enzymatic activities of Coprinoid mushrooms are however still insufficiently studied.

Different levels of milk-coagulating abilities in *Coprinellus micaceus*, *C. disseminatus*, *C. xanthothrix*, *Coprinopsis cinerea*, *C. radiata*, and *C. strossmayeri* were detected in our studies. During mycelial cultivation, proteolytic enzymes are secreted into cultural broth. Proteolytic enzymes also accumulate in fruiting body tissues. Proteolytic activities of malt- extract culture liquids and of mycelium of various species were investigated for their proteolytic activity on defatted milk by coagulation and peptonization tests after 5 days of incubation.

Tested culture liquids showed different degrees of proteolytic activities but there was no significant correlation to fungal cultivation times. However, the doze/effect correlation was noted. Strong proteolytic activity was revealed in culture liquids from *C. micaceus, C. xanthothrix* and *C. cinerea* starting from the first day of incubation whereas mycelial extracts of these species were inactive. A weaker activity compared to culture liquid samples was detected. In contrast, in mycelial extracts of *C. radiat‡, C. disseminatus* and *C. strossmayeri* proteolytic activities were detected that were however weaker than the activities in the culture liquids.

This work is supported by grants from the NATO (#980764) and the DAAD (#548.104401.174) grants and by the Deutsche Bundesstiftung Umwelt (DBU).

PS4-429-0573

The histidine kinase Dic1p regulates HOG1-MAPK involved in glycerol accumulation of Cochliobolus heterostrophus.

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In Southern corn leaf bright fungus *Cochliobolus heterostrophus*, the histidine kinase Dic1p is involved in resistance to dicarboximide and phenylpyrrole fungicides. We previously reported that the phenylpyrrole fungicide fludioxonil and the dicarboximide fungicide iprodione misled to activation of Hog1-type MAPKs in some phytopathogenic fungi including *C. heterostrophus*. To elucidate the relationships and functions of *C. heterostrophus* BmHog1p (Hog1-type MAPK) and the histidine kinase Dic1p, the phosphorylations of BmHog1p and glycerol-accumulation were analyzed in the wild-type, the *dic1* deficient and the *Bmhog1* deficient strains. In the wild-type strain, the phosphorylated BmHog1p was detected after exposure to both iprodione and fludioxonil, even at concentration of 1 µg/ml. In the *dic1* strain, no phosphorylated BmHog1p was detected after exposure to 1 µg/ml and 10 µg/ml of the fungicides. Similarly, in response to osmotic stress (0.4 M KCl), a phosphorylated BmHog1p was also not found in the *dic1* strain, whereas the band representing the active BmHog1p was clearly detected in the wild-type strain. The treatments with iprodione and the osmotic stress caused intracellular glycerol accumulation in the wild-type strain but not in *dic1* and *Bmhog1* strains. These observations suggested that the Dic1 histidine kinase positively regulates Hog1-type MAPK, resulting glycerol accumulation for osmotic adaptation of *C. heterostrophus*.

PS4-430-0581

Interspecific interactions between saprotrophic basidiomycetes: effect on gene expression and chemical activity of mycelia.

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Basidiomycete saprotrophs are the major agents of decomposition in woodland ecosystems. Their high abundance frequently leads to interactions, both between and within species, as they come into contact and compete for space and resources. Very little is known about which genes and metabolic pathways are involved in these interactions, and how they influence their outcome. This project aims to identify differentially expressed genes and chemicals produced during interactions.

A subtractive cDNA library was constructed for the interaction between *Trametes versicolor* and *Stereum gausapatum*. By using subtractive hybridisation (SSH) only those genes that are unique to interactions have been isolated. cDNA microarrays of this library were used to identify genes that are up and down regulated during the interaction. Further experiments using these arrays studied interactions between *T. versicolor* and other species, which have different outcomes to find if different genes are active in different circumstances.

The other vein of the project looks at the volatile organic compounds (VOCs) produced by these interacting fungi using solid phase microextraction (SPME) and GC-MS, diffusible chemical production using HPLC and enzyme activity using histochemical staining techniques.

The arrays have shown many genes to be up-regulated during the interactions with some differences observed during the interactions between different species. The expression of a number of these genes is being verified using RT-PCR. Full analysis will be complete by the time of the conference.

GC-MS work has identified several compounds unique to interactions, including several sesquiterpenes. Previous studies have found them to be important as insect attractants and antifeedants.

Histochemical staining showed increased production of peroxide and superoxide in interaction zones of interspecific interactions when compared with the rest of the colony and self-pairings.

This work is one of the first studies to look at the molecular basis of interactions between basidiomycetes using subtractive hybridisation. The arrays present the opportunity for probing with many different species interactions and have the potential to shed light on the mechanisms driving these interactions. We also have 8000 clones we hope will be sequenced in the very near future. This transcriptomics work, together with the metabolomics has given us a well rounded picture of what's going on during these interactions and presents some exciting results. There is great potential to build on these results and extend the work further and expand the approaches to include proteomics to give a systems biology perspective on these important interactions.

PS4-431-0588

Characterization And Frequency Of Mating Type Genes In Cercospora

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The genus *Cercospora* consists of numerous important plant pathogens. Approximately 600 *Cercospora* species are currently recognized, with a further 281 morphologically indistinguishable species placed in the *C. apii sensu lato* complex. Known teleomorphs of *Cercospora* belong to the genus *Mycosphaerella*, but for most *Cercospora* species the teleomorph is unknown. During this study, degenerate primers designed from homologous areas in related species were used to amplify part of the mating type genes of *C. beticola* from sugar beet, *C. zeae-maydis* and *C. zeina* from maize, and *C. apii* and *C. apiicola* from celery. Chromosome walking was used to sequence the full length mating type genes of these *Cercospora* species. The nucleotide and protein sequences were compared to that of other fungal mating type sequences and it was found that the structural organization of the *Cercospora* mating type genes are similar to that of other ascomycetes. The full length *Cercospora* sequences were used to develop primers for the amplification and sequencing of homologous portions of the *MAT1-1-1* and *MAT1-2* genes of additional *Cercospora* species. Phylogenetic analyses of these sequences revealed little variation among the species belonging to the *C. apii* complex, whereas the mating type genes of *C. zeae-maydis* and *C. zeina* were found to be dissimilar. The presence of the mating type genes was determined for field populations of *C. apii*, *C. apiicola*, *C. beticola*, *C. zeae-maydis* and *C. zeina*. The two mating types were found to be present in approximately equal proportions in populations of *C. beticola*, *C. zeae-maydis* and *C. zeina*, suggesting that these species are heterothallic and that the sexual cycle could be active. This, however, was not the case for the two species occurring on celery, as all isolates of *C. apii* were found to contain *MAT1-1-1* and all isolates of *C. apiicola* to contain *MAT1-2-1*. This study represents the first characterization of mating type genes for species of the genus *Cercospora* and only the third for the genus *Mycosphaerella*.

PS4-432-0600

Enzymatic activity of endophytic fungi on leaf decomposition

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Fungal endophytes and saprobes on leaves of *Magnolia liliifera* were investigated. Nine endophytes were existed as saprobes during leaves decomposition. The phylogenetic examination was also confirmed that each counterpart of endophytes and saprobes taxa are same species. Probably endophytes change lifestyles on host senescence and colonized as saprobes. It would be interesting (1) to verify the pattern of degrading enzyme production during the leaf decay, (2) to determine the ability of enzyme production in liquid medium compare among endophytes and saprobes which are same species. Fresh senescent leaves of *M. liliifera* were treated as the test group and divided into 2 groups, and incubated in trays covered with gauze. The trays were placed outdoors and sprayed with sterile water every day. The leaves were collected over a period of 88 days and assays for cellulase, xylanase, mannanase, polygalacturonase and laccase. Sterilised leaves were used as control. (2) Nine endophytes and 9 saprobes were culture in liquid basal medium supplemented with carbon source suitable to each enzyme production. Enzyme activity was assay from crude extract. A succession in enzyme production starting with xylanase, followed by mannanase and finally cellulase was observed. Mannanase had the highest activity. All endophytes and saprobes had high capability to produce mannanase in liquid medium. Seven endophytes did produce xylanase, 3 endophytes did produce cellulase, 5 endophytes produced polygalacturonase and 1 endophyte produced laccase. The ability of endophytes to produce carbohydrases may reflect their role in nature in degrading dead leaves. Endophytes within *M. liliifera* leaves have the ability to produce carbohydrases and existed as saprobes follow leaves senescence.

PS4-433-0601

How do endolithic lichens dissolve carbonates?

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Endolithic lichens are a peculiar group of organisms that live embedded in the substratum. They actively dissolve the carbonates by means of an unknown metabolic mechanism, purportedly related to the release of respiratory CO2 and/or the secretion of specific organic acids, e.g. oxalic acid. In this contribution some new findings are reported, clearly supporting the hypothesis that a more sophisticated mechanism, i.e. the secretion of specific enzymes that modify the complex CO2/H2CO3/CaCO3 equilibria, is involved in the substratum dissolution. Nine species, selected among the most common endolithic lichens of north-eastern Italy and characterized by different ecology, geographic distribution and photobiont (*Acrocordia conoidea, Caloplaca alociza, Clauzadea immersa, Petractis clausa, Protoblastenia calva, Rinodina immersa, Verrucaria baldensis, V. hochstetteri, V. marmorea*), were studied in detail. FT-IR analyses demonstrated that, in opposition to epilithic calcicolous lichens, none of the studied endolithic lichens accumulate Ca oxalates in detectable quantity. Calcite is the most common biomineralisation product: dissolved near the most active parts of the lichen thallus, it re- precipitates in the lithocortex. Histological, biochemical and biomolecular techniques applied to thalli still immersed in the substratum, or free from it showed that all the studied species produce specific carbonic anhydrases (CA), that are thus reported for the first time from lichenised ascomycetes. These enzymes are particularly frequent in the lithocortex and in the oil-hyphae of the pseudomedulla. CA are important for several fundamental metabolic processes, from CO2 transport to acid-base balance, because they catalyze the reversible hydration of CO2. CA might play a significant role in the substratum dissolution, because their secretion in the sites of active growth would significantly intensify the chemical activity of respiratory CO2, whereas their presence in the hyphae would favour lipogenetic processes, explaining the high content in triacilglycerols typical of endolithic lichens. To verify these hypotheses, experiments are now extended to axenic cultures of selected apomycobionts in order to study *in vitro* the interactions between hyphae and calcite crystals, and to verify the secretion of CA or organic acids.

PS4-434-0602

Endolithic lichens on carbonatic rocks: biodeterioration or bioprotection?

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Calcareous outcrops represent c. 70% of the exposed stone surfaces of the Earth. In Europe c. 60% of them are colonized by organisms, the most common being the so-called endolithic lichens, that live embedded in the substratum, and actively dissolve the carbonates by means of a poorly known metabolic mechanism. It is not clear whether a rock surface that is covered by endolithic lichens weathers at a slower or a faster rate than an identical but organism-free surface. According to some authors, the mean lowering rate of the former typology would be orders of magnitude greater than bare surfaces. Others, on the contrary, state that especially in longer terms, the presence or absence of organisms would have made little difference to overall rates of rock weathering. Unfortunately, no quantitative data are available for a sound comparison. In order to clarify the bioweathering vs. bioprotective role of these organisms, an extensive series of field measurements were recently undertaken by the authors with the main aim of quantifying the lowering of limestone surfaces in a wide array of different environmental conditions. *In situ* measurements are being carried out with a traversing-microerosion meter (t-MEM; estimated precision: 1 µm) in eighteen sites selected along two elevation gradients from 0 to 2500 m, along the line Trieste Karst – Mt. Canin (south-eastern Alps), and in the Maiella Massif (Central Italy). In each site 6 measuring stations were selected on horizontal, smooth limestone surfaces colonized by endolithic lichens. Two further stations consisted of not colonized rock surfaces exposed by mechanical break and cutting, and re-exposed horizontally. One sample of the lichens colonizing each measuring station was taken for identification, and thin and polished sections were prepared to describe petrography and the most typical bioweathering phenomena caused by endolithic lichens. The first results of this research will be thoroughly discussed on the basis of a 20-years-old series of similar observations carried out in the calcareous areas of north-eastern Italy.

PS4-436-0673

Highest thermostable xylanase produce from newly isolates Thermomyces lanuginosus strains

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Xylanases have significant current and potential uses for several industries including paper and pulp, food, and biofuel. High productivity and thermostability were desirable for the application in this enzyme. Eighty-eight isolates of *Thermomyces lanuginosus* were isolated from samples collected from various ecological systems at different geographical regions of Thailand. All strains could produce cellulase-free xylanases with diversified ability on productivity and thermostability. This paper describes the highest thermostability of-xylanase produced by newly isolate of *T. lanuginosus* strain THKU-49 and effect of ionic strength on thermostabilty. Purified xylanase from this strain showed high thermostable xylanase having half-life 1,266 minutes at 70^{0}C, pH 6.0 that was higher than other reported in same species. The enzyme was unstable at pH 5.0 and lost all activity after 30 min incubation without present of substrate at 70oC. The optimum temperature and pH of the enzyme were determined to be temperature 70oC and pH 6.0, respectively. The *Vmax*, *Km* and thermal inactivation values were 1,225 mmol/min/mg proteins, 9.1 mg/ml and 26kJ/mole, respectively. The molecular weight of b-xylanase protein estimated by SDS-PAGE was 24.9 kDa. Type and ionic strength of buffer had the effect on thermostability of the purified enzyme. The enzyme in 3-400 mM phosphate buffer (pH 6.0) was stable than in citrate buffer at the same concentration. The highest half-life of the enzyme was found at 3 mM. When buffer concentration increased, the half-life was significantly decreased. The effect of ionic strength on thermostability was more significant at higher or lower than pH 6.0.

PS4-437-0680

The enticing odour of fungi – A safety thread, tying insect-fungal associations?

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Insect-fungus associations are diverse from mutualism to symbiosis. In wood inhabiting beetles, a continuum of associations from dispersal to true "gardening" can be found. Both involved organisms evolved morphological adaptations which guarantee dispersal of the fungus. We analysed fungal odours in an insect-fungus symbiosis and we found a strong influence on insect behaviour triggered by volatile metabolites of the ectosymbiont. The large timberworm (*Hylecoetus dermestoides* L., Lymexylidae) is a beetle whose larvae are xylomycetophagous in various tree species. In our chemoecological studies we examined the odour of suitable and infested wood trunks by gaschromatographic and mass spectrometric analysis of volatile organic compounds released by bark and wooden tissue. Their perceivability by the insect antenna was simultaneously examined by electroantennographic recording (GC/MS-EAD) and behavioural activity of perceivable components was tested in field trap experiments. A single compound turned out to be highly attractive to females on host-search flight. The odour analysis of several fungal species associated with *H. dermestoides* yielded the origin of this particular isoprenoid. It is produced in high amounts by the ectosymbiotic fungus of *H. dermestoides*, the ascomycetous yeast *Ascoidea hylecoeti* (Batra & Francke-Grosmann), both growing in the larval galleries of wooden trunks and on artificial culture media. The larval stage only feeds on this fungal ectosymbiont. Specific genitalian fungus pouches of the very short-living females assure the transfer of exclusive *A. hylecoeti* to their offspring. The fact that female imagines, searching for egg deposition sites are strongly attracted to the odour of their own symbiont is remarkable. Two explanations are discussed:
1. Wood trunks are sparsely distributed but suitable hosts for several years. The fungal isoprenoid compound serves as an attractive semiochemical for the insect (like an aggregation pheromone) for the following generation.
2. The attraction to an *A. hylecoeti* specific compound is a persisting ancestral relict which was necessary for evolutionary development of the mycetome carrying the fungal symbiont of *H. dermestoides*.
Furthermore a functional role in symbiosis, the perpetuation of the insect-fungus dependence is imaginable, but remains speculative.
As shown in our example, chemoecological methods can provide remarkable insights into insect-fungal associations. Function of fungi might be identified by this chemotaxonomic data. Evolutionary studies on the spectrum of interaction in this system seems promising.

PS4-439-0746

Cloning and characterization of mating related genes in *medicinal Ganoderma lucidum*

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Ganoderma lucidum is a tetrapolar mushroom of medicinal value. In this study, we attempt to elucidate the genes possibly involved in mating behavior via molecular approaches. A set of mating homologous genes identified in *Coprinus cinnereus* and *Schizophyllum commune* genome was used to blast against the previously constructed, assembled, and annotated genomic shotgun and cDNA EST library of *G. lucidum*. At least four genes: a1, a2, b1, b2 encompass homeodomain protein HD1 and HD2 in A1 mating locus; 20 pheromone precursor and 19 pheromone receptor genes in B mating locus were accessed. The a1, a2 genes in A1 mating type have been cloned and their full-length cDNA resolved, each with 1428 bp and 1680 bp respectively. The homeodomain HD1 encompassed a putative nuclear localization signal (NLS) domain PKRRR and exhibited 48% similarity to the HD1 of *Pleurotus djamor*; HD2 encompassed a NLS domain, RRSRCRKE, and exhibited 39% similarity to the HD2 of *Pleurotus djamor*. Two binary vectors PCG1-a1 and PCG1-a2, harboring a1 or a2 gene insert and driven by glyceraldehydes-3-phosphate dehydrogenase (gpd) promoter with the marker hygromycin resistance gene were constructed and scheduled to transform monokaryotic compatible mating strain by electroporation to resolve their function.

PS4-440-0837

Nitrogen enrichment promotes phosphatase activity in *Cladonia portentosa*

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The common heathland lichen *Cladonia portentosa* (Dufour) Coem. expresses both acid phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activity. A capacity to utilise the organic fraction of phosphorus available in atmospheric deposits may confer an ecological advantage to *C. portentosa* growing under nutrient-limiting conditions. Nitrogen enrichment of oligotrophic habitats can lead to increased plant demand for phosphorus. Previous studies on *C. portentosa* have demonstrated a strong covariance between thallus nitrogen and phosphorus concentrations when samples from sites across the UK subject to different N deposition loads were compared. Therefore we investigated the relationship between N enrichment and phosphomonoesterase activity in the apices (top 10 mm) of this common heathland lichen. Under field conditions at Whim Bog, Central Scotland, *C. portentosa* cushions were subject to either NO3- or NH4+ treatments at 8 (control), 16, 32 and 64 kg N ha-1 yr-1. The effect of artificially elevated deposition of both P and K was also investigated. There was a significant increase in PMEase activity with increasing N deposition (as either NO3- or NH4+) suggesting that as N supply increases, *C. portentosa* has the capacity to allocate an increasing quantity of N to phosphatase synthesis. This enhanced enzyme activity might explain the observed relationship between thallus N and P concentrations. Phosphomonoesterase activity was not stimulated in treatments receiving P and K in addition to N; this was interpreted as an effect of increased availability of inorganic P. It was concluded that N enrichment promotes phosphatase synthesis and phosphorus capture in *C. portentosa* thus maintaining N:P stoichiometry.

PS4-441-0886

Fatty acid beta-oxidation pathways during Magnaporthe pathogenesis

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Lipid metabolism is a key pathway during development in different organisms. In mammals, beta-oxidation of fatty acids takes place in both the peroxisome and the mitochondria, unlike in yeasts where it is an exclusive peroxisomal function. Recently, in the filamentous fungi *Aspergillus nidulans*, in addition to the peroxisomal component, mitochondrial beta-oxidation has been shown to be required for complete fatty acid catabolism. We used mutation analysis to characterize the function of specific beta-oxidation enzymes associated with the mitochondria (Mg*ECHA*) and the peroxisomes (Mg*FOX2*). The inability of either single mutant to grow on fatty acid medium demonstrates that both the peroxisome and the mitochondria contribute to lipid metabolism. Both deletion strains are defective in the penetration of the host epidermis. Exogenous addition of high concentrations of glucose restores normal appressorial morphology and partially restores host penetration. Taken together this suggests that both mitochondrial and peroxisomal beta-oxidation function during early pathogenesis. The breakdown of lipid bodies provides the necessary input for primary and secondary metabolism before host penetration. Preliminary data on the specific contributions of mitochondrial and peroxisomal fatty acid oxidation during pathogenesis shall be presented.

PS4-442-0904

Characterization of disruption mutant of phosphoinositide-specific phospholipase C gene plcA in the model filamentous fungus *Aspergillus nidulans*

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Phosphatidylinositol-specific phospholipase C (PLC) is a family of enzymes that hydrolyze phosphatidylinositol 4,5-bisphosphate into two well-established second messenger molecules, diacylglycerol and inositol 1,4,5-trisphosphate. Thus, PLCs perform essential roles in the signal transduction pathways triggered by diverse environmental cues such as growth factors, hormones, neurotransmitters and antigens. In the model filamentous fungus *A. nidulans*, the genome sequencing has revealed the presence of four putative PLC genes: *plcA* gene (chromosome VIII) encoding AN0664.2; *plcB* (VI) for AN2947.2; *plcC* (I) for AN6382.2; and *plcD* (II) for AN3636.2.
In this study, *plcA* gene encoding the putative PLC AN0664.2 was disrupted by marker replacement and the disruptant was characterized with respect to their growth properties to get an insight into the physiological function of the gene product in the fungus.
The spores of mutants disrupted in *plcA* gene showed normal swelling but marked impairment and delay in germ tube emergence at 25?. They remained as swollen spores without germ tubes until 16 hrs after incubation in culture medium, while the spores of RMS010 wild type strain had germ tubes 5-10 times as long as the swollen spore diameter under the same conditions. The defect was much less conspicuous at higher temperatures. When the spores of wild type and deletion mutant strains were allowed to germinate at 37^0 and then incubated at 25^0, hyphal growth of the dirsuptant was slower than that of RMS010 strain, but the difference was much less dramatic.
The phenotypes shown in deletion mutant suggests that one of the roles of *plcA* in *A. nidulans* is in the redirection from the initial spherical growth (swelling) to polarized growth i.e. germ tube emergence.

PS4-443-0905

At what age becomes *Cliostomum corrugatum* adult?

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The objective was to investigate at what the age specimen of *Cliostomum corrugatum* become fertile in order to estimate the time span between the meiosis events. The species has its main distribution in Europe but has also been found on the west coast of British Columbia and is red listed, e.g., in Sweden (nearly threatened), Denmark, Germany and England. In the province of Östergötland, southern Sweden it is most frequent on old *Quercus robur* trees in open oak forest or meadows. I may also be found on other deciduous trees as *Ulmus* and *Fraxinus* species. It is mainly groving on the flat terminal parts of the rough bark of the tree trunks and not on the sides of the cracks.
Cliostomum corrugatum does not grow on young oak trees. The smallest tree trunk diameter with *Cliostomum corrugatum* was is 0.65 m, a tree of at least 100 years of age. On two localities in Östergötland all oaks were studied and the size of the trees and the size of the largest thallus of *Cliostomum corrugatum* were recorded. Out of this data the size of how small a tree can possibly be for hosting *Cliostomum corrugatum*. This estimate was compared with the size of the smallest thalli with apothecia and the size of trees on which these appeared. With knowledge of the peripheral secondary growth of oaks it was possible to estimate the age of the youngest fertile *Cliostomum corrugatum* to about 30 years. Thus, equal to the time span between two meiosis events. .

PS4-444-0906

Analysis of cellobiohydrolase, pectinase, and xylanase in *Trichoderma* fungi using chromogenic media

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Trichoderma is one of well-known fungal groups that produce useful enzymes for agricultural and industrial application. Many *Trichoderma* fungi have been isolated in Korea for several decades. But their ability of producing enzymes has not been evaluated yet. This work was carried out to evaluate 145 Korean isolates of thirty-four *Trichoderma* species for their ability of degrading cellobiose, pectin, and xylan using chromogenic media. Tests with 4 different chromogenic-dyes, congo-red, phenol red, remazol brilliant blue and tryphan blue showed that congo-red was the most useful dye in the clear detection of cellobiohydrolase, pectinase, and xylanase, and the optimum pH condition of the congo red chromogenic media was pH 7.0. The activity of cellobiohydrolase in *Trichoderma* was generally strong in all the *Trichoderma* isolates tested. *T. reesei, T. harzianum, T. pseudokoningii*, and *T. aureoviride* showed the higher ability of producing cellobiohydrolase than other tested species. Pectinase activity was detected in 57 *Trichoderma* isolates. *T. reesei, T. harzianum, T. pseudokoningii*, *T. aureoviride, T. cf. virens* and *T. longibrachiatum* showed strong pectinase activity. In xylanase assay 45 *Trichoderma* isolates showed activity. Strong xylanase activity was detected from *T. reesei, T. harzianum, T. minutisporum*, *T. virens* and *T. longibrachiatum*. Overall, it was found that *T. reesei* and *T. harzianum* are the most active enzyme producing species in the 34
Trichoderma species tested.

PS4-445-0910

Expression patterns of a gene involved in secondary metabolism from the endophyte, *Epichloë festucae*

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Fungal endophytic species of the genera *Epichloë* form mutualistic symbioses with cool season grasses of the family Pooideae. *Epichloë festucae* produces a variety of secondary metabolites including lolitrem alkaloids, specifically lolitrem B, a deterrent against grazing animals. The gene cluster involved in the biosynthesis of lolitrems (*ltm* genes) has been isolated and characterised from *E. festucae* with ten genes identified as part of the cluster (Young et al., 2005, Young et al., 2006). Expression of these genes has not been observed in axenic cultures of *E. festucae* isolates grown on potato dextrose agar but are highly expressed *in planta*. To gain an understanding of where and when these genes are expressed *in planta*, several P*ltmM-gusA* constructs were prepared and transformed into *E. festucae*. Associations between perennial ryegrass (*Lolium perenne*) and these transformants were established and GUS expression analysed. The minimum promoter length required to drive *gusA* expression was 800 bases. In vegetative tillers, reporter gene analysis indicated that the *ltm* genes were being expressed at all times in all aerial parts of the plant and in epiphyllous hyphae. Spikelets from reproductive tillers were analysed for reporter gene expression at both pre-anthesis and post-anthesis developmental stages. At pre-anthesis, reporter gene expression was observed largely in the rachis, rachilla, glumes, lemma, palea and, occasionally, the anthers. In post-anthesis spikelets, reporter gene expression was observed almost solely in the gynoecium. Germinating seeds and seedlings were examined for reporter gene expression. Staining was observed in germinating seeds 24 hours post-germination but not in seedlings older than 2 days and younger than 6 days post-germination. After day 6, hyphae exhibited gene expression from the mesocotyl to the tip of the emerging plumule.
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PS4-446-0911

Pathogenic Fungi on Horse Chestnut Trees– Volatile Organic Compounds as a Tool to Study Ecological Interactions

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Leaves of the Common Horse Chestnut *Aesculus hippocastanum* and the Red Horse Chestnut *Aesculus* x *carnea* are attacked by the pathogenic fungi *Guignardia aesculi* (Botryosphaeriaceae) and *Erysiphe flexuosa* (Erysiphaceae). Leaves infected by the endoparasite *G. aesculi* show necroses, start rolling in, and die with progressive development of fungus. The mycelium of the ectoparasitic powdery mildew *E. flexuosa* covers the leaf surface and stalks with a greyish white coating. In contrast to *G. aesculi*, infection of *E. flexuosa* does not cause serious necroses on leaves.
The degree of infection by the pathogens on leaves of *A. hippocastanum* and *A.* x *carnea* was examined in field studies throughout the year. The infection increased during the season and both diseases could grow in parallel at the same leaves. In autumn *E. flexuosa* could cover the whole leaf surface on examined trees. Even on necroses of *G. aesculi* the mycelium of the powdery mildew was visible.
Pattern of volatile organic compounds emitted by healthy leaves and leaves infected by one or both fungi were identified using gas-chromatography coupled with mass-spectroscopy (GC-MS). Compared to healthy leaves, pathogenic infection induced the release of additional volatile organic compounds. There were no differences in induced compounds emitted from the different horse chestnut tree hybrids. Leaves infected by the pathogens released fungi related volatile compounds as 1-octen-3-ol, 3-octanone and 3-octanol. The composition of these C8-compounds was depending on species infecting the leaves. Another volatile compound in the headspace of infected leaves was benzyl alcohol. The origin of this compound, i.e. fungal volatile, plant response or fungus-fungus competition has to be examined in further studies.

PS4-447-0912

The Enticing Odour Of Fungi – The role of Volatile Organic Compounds for a xylomycetophagous beetle and its fungal associates

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In galleries of wood inhabiting and fungal-feeding beetles a typical pattern of associated fungi can be found. Whereas some fungi are strongly bound to their insect symbiont and maybe transported actively in special pouches or organs, others can be transported by sticking on the surface of beetles. The latter relationship seems to be mainly mutualistic but is still subject of considerable debate.
As a first step to enlighten the fungus-insect-association we examined volatile organic compounds (VOC) released by different fungi found in galleries of the large timber worm (*Hylecoetus dermestoides* L., Lymexylidae), a xylomycetophagous beetle. The main fungal symbiont, *Ascoidea hylecoeti* (Batra & Francke-Grosmann) is transported actively in specific fungal pouches of the female to ensure infestation of galleries inhabited by the larva. Several other fungal species can be found in the galleries regularly (*Ophiostoma bacillisporum & Ceratocystis torulosa*, Butin & Zimmermann), others rarely occur (e.g. *Graphium penicilloides*, Corda). We examined the odour of wood tissue and artificial media infested with several of fungi. Volatile chemical compounds produced by each fungus were determined and tested for perceivability by the insect antenna by coupled electroantennographic recording (GC/MS-EAD). Their behavioural activity was tested in field trap experiments. The strength of attraction to different compounds and their occurrence in the different fungi species is discussed. These chemotaxonomic data are used to approach the diversity of associations in this insect-fungus community.

PS4-448-0941

Comparison of physiological characteristics between environmental and clinical isolates of human pathogenic *Pythium insidiosum*

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Pythium insidiosum is the etiological agent of pythiosis, a life-threatening infection in mammals. Human pythiosis is generally acquired through zoospore attachment, while standing in swampy areas or fields. Recently, we have isolated *Pythium insidiosum* from irrigation water in the provinces in northern region of Thailand. In this study, physiological characteristics of *Pythium insidiosum* environmental- and human-isolates were determined. The investigation has been performed with 19 environmental isolates and 10 human isolates of *Pythium insidiosum*. All isolates showed broad, sparsely septate hyaline hyphae with perpendicular branching. They produced filamentous sporangia in induction medium. Seventy-six percent of natural isolates and sixty percent of clinical isolates showed peak growth rate at 30^{0}C. Twenty-four percent of natural isolates and forty percent of clinical isolates reached peak growth rate at 37^{0}C. Immunogenic profiles of both environmental and clinical isolates were determined using immunoblotting analysis. Most common reactive bands of both environmental and clinical isolates have been indicated at the molecular weights ranging from 35-38 kDa. Immunoblot reactivity of two closely related water molds was examined. *Pythium grandisporangium* showed very weak reactive bands, while *Pythium cystogenes* displayed no immunoreactivity against rabbit antiserum immunized with antigens of *Pythium insidiosum*. The growth characteristics and immunoblotting patterns of environmental isolates are similar to those observed in clinical isolates. From these results, it has been suggested that *Pythium insidiosum* living in irrigation water is likely to be the potential causative agent of pythiosis in high-risk group of people, especially farmers and persons who work continuously in agricultural areas.

POSTER SESSION 8 POPULATION GENETICS

PS8-449-0037

Intersterility Groups Of *Pleurotus* In Iran

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Oyster mushroom genus *Pleurotus* are among the most commonly known groups of saprophyte, edible and medicinal fungi. A taxonomic confusion has always been associated with the genus *Pleurotus* in last years. In Iran, *Pleurotus* species are widely distributed throughout the country and are a well known edible mushroom and cause of wood decay. The taxonomical position of Iranian populations of *Pleurotus* and compatibility reactions has never been clearly understood. In this study, Haploid and dikarion cultures were paired in all of the possible "haploid-haploid" and "haploid-dikarion" combinations. Sexual compatibility was determined based on presence or absence of clamp connections. From the results of pairing tests, isolates were grouped into five intersterility groups. Isolates of intersterility groups were paired with tester strains and results showed that Iranian intersterility groups are compatible with *P. ostreatus*, *P. cornucopiae* and *P. pulmonarius*, *P. calyptratus*, and *P. eryngii* species. Morphological characteristics used to distinguish species of the *P. ostreatus* Complex, were not useful when applied to corresponding mating compatible groups from Iran.

PS8-450-0048

Population diversity of *Fusarium proliferatum*, as the major causal agent of rice bakanae disease in Mazandaran province, using vegetative compatibility groups

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Fifty three isolates of *Fusarium* species belonging to section *Liseola* were established from bakanae infected rice plants from different localities in Mazandaran province during 2004. Genetic diversity of *F. proliferatum* as the major causal agent of rice bakanae disease in Mazandaran province was investigated. Nitrate non-utilizing (*nit*) mutants were generated from 35 isolates in order to study vegetative compatibility among these isolates. Five hundred seventy four *nit* mutants were obtained using PDC and MMC culture media containing 3% Potassium Chlorate. *Nit* mutants were divided into three phenotypic classes (*nit1*, *nit3*, and *NitM*) based on their growth on the medium containing different nitrogen sources. Of the obtained *nit* mutants, 91/81%, 5/57%, and 2/61% were *nit1*, *NitM*, and *nit3*, respectively. *Nit* mutants obtained from six isolates were *nit1*, only. These isolates were discarded. *Nit* mutants were used to force heterokaryon to determine distribution of vegetative compatibility groups (VCGs). We placed 29 strains into 26 vegetative compatibility groups (VCGs). Of the 26 VCGs identified, 23 (VP4-26) were represented each by a single isolate and remaining 6 belonged to three (VP1-3) multimember , each containing two strains, VCGs. VP3 is limited to strains from the same field. VP1 and VP2 each contained strains from two fields in different localities. All multimember VCGs were placed in special limited area indicating more similarity of this area's population than other areas.

PS8-451-0064

GLOBAL migration patterns in the fungal wheat pathogen *Phaeosphaeria nodorum*

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The global migration patterns of the fungal wheat pathogen *Phaeosphaeria nodorum* were analysed using twelve microsatellite loci. Analysis of 693 isolates from nine populations indicated that the population structure of *P. nodorum* is characterized by high levels of genetic diversity and a low degree of subdivision between continents. To determine whether genetic similarity of populations was a result of recent divergence or extensive gene flow, the microsatellite data were analysed using an isolation with migration model. We found that the continental *P. nodorum* populations diverged recently, but that enough migration occurred to reduce population differentiation. The migration patterns of the pathogen indicate that immigrants originated mainly from populations in Europe, China and North America.

PS8-452-0065

ORIGIN and divergence of the fungal pathogen *Mycosphaerella graminicola;* from wild grasses to domesticated wheat

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The Fertile Crescent represents the center of origin and earliest known domestication for many agricultural crops. During the transition from wild grasses to domesticated cereals, many host-specialized pathogen species are likely to have emerged. A potential ancestral species of the wheat-adapted pathogen *Mycosphaerella graminicola* was identified on wild grasses collected in Northwest Iran. Isolates of this wild grass pathogen from five locations in Iran were compared to 123 *M. graminicola* isolates from four geographical populations. ITS sequencing revealed a close phylogenetic relationship between the two pathogen species. To reconstruct the evolutionary history of *M. graminicola* we used information from six DNA loci encompassing 392 SNPs. Coalescence analyses suggested a relatively recent origin of *M. graminicola,* during the same time frame as the domestication of wheat in the Middle East 11,000 years ago. Our estimates of gene flow are consistent with an extensive expansion of *M. graminicola* throughout the world, with no evidence that gene flow occurs between pathogen populations on wheat and wild grasses at the present time.

PS8-453-0075

Population genetics of the basidiomycetous fungus *Pleurotus pulmonarius* based on haploid-dikaryotic life cycle

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Fungal populations are shaped through the process of sexual and asexual reproduction, growth and interactions between individuals. Population analysis of the basidiomyceteous fungus *Pleurotus pulmonarius* collected in three regions of Central Russia – Moscow, Voronezh, and Tver – was performed. Since *P. pulmonarius* is characterised by a haploid-diploid life cycle, genetic analysis was carried out on dikaryons (diploids) and homokaryons (haploids) simultaneously. Both homokaryotic and dikaryotic strains (parents – offspring) can be easily reproduced on standard media, either agar plates or liquids. In other words, the diploid parent and its haploid progeny can be analysed in the same experiment, and the fungus complete life cycle ('from spore to spore') can be reproduced in the laboratory. Most of the basidiomycete fungi are known to form panmictic populations resulting from the haploid progeny hybridisation. Bifactorial multiallelic sexual compatibility system is presumed to provide high level of panmixia. However, this reproduction mode also implies 25% of self-matings that could result in the relatively high inbreeding rate in natural populations.

To estimate the outbreeding rate and mechanisms which shape the *P. pulmonarius* populations, we employed the analysis based on mating type factors distribution and molecular markers (allozymes, RAPD). To define interbreeding fungal populations, crosses between homokaryons were performed. Besides, genetic exchange in basidiomycetes is not only limited to the sexual cycle. Genetic recombination is shown to occur in heterokaryon-homokaryon mating (he-ho-mating), i.e. a heterokaryon is capable to contribute fertilising nuclei to the haploid homokaryon resulting in novel heterokaryon. Reproductive barriers themselves prevent hybridising between haploid (homokaryotic) mycelia differed at mating type loci that is resulted eventually in developing intersterility groups. Intersterility groups limit gene flow between sympatric populations.

Somatic incompatibility between genetic individuals (genets) in *P. pulmonarius* was performed as a distinct antagonistic response ('barrage') that prevented free exchange of nuclei and cytoplasm, while anastomosis between genetically identical clones were resulted in perfect fusion. Somatic clones were propagated only on the same substrate (log). They were identical at allozyme loci and RAPD profiles.

The mean genetic diversity for all populations studied was high (I = 0.824). Genetic differentiation among *P. pulmonarius* populations was supported by F-statistics (FST = 0.750). The low level of inbreeding (FIS = 0.018) suggests that the *P. pulmonarius* populations are panmictic, and the main reproduction mode involves basidiospore dispersing at long distances. However, gene flow between geographic localities is restricted. Geographically isolated populations and intersterility groups were clearly differentiated within the species.

The research was supported by RFBR grant.

PS8-454-0187

Variability in the IGS1 region of *Rhodocollybia laulaha*

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In order to investigate microevolutionary processes particular to the macrofungi, a population-level study of the Hawaiian endemic mushroom *Rhodocollybia laulaha* is being conducted. The first goal is to identify nuclear markers that provide substantial resolution to discern patterns within the *R. laulaha* populations of the Hawaiian Islands. The IGS1 (nuclear ribosomal inter-generic spacer) region can be useful as a genetic marker that tracks genets within fungal populations and should be a good candidate marker for discerning intra-population level pattern. For the current project, IGS1 sequence data was generated for 11 collections of *R. laulaha*. Additionally, multiple clones of the IGS1 region were sequenced for four of those 11 collections to compare intra-collection variation (between paralogous copies of the IGS1 in a single genome) and inter-collection variation (between the IGS1 regions of different mushrooms in the same species). Of 510 base pairs aligned, only 32 sites were variable (22 substitutions and 10 indels). The observed variation could be due to the presence of various alleles for the IGS1 and/or variations between paralogous copies. Issues associated with PCR-mediated recombination, PCR-Taq error, and inefficiency of concerted evolution will be discussed as possible agents leading to this variation.

PS8-455-0208

Genetic Variations of *Tricholoma crassum* in some Areas of Thailand by Isozyme Electrophoresis and PCR-RFLP Method

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Trichloma crassum is an large and good edible species that found growing abundantly throughout Thailand. One hundred and thirty-eight monokarytic isolates of *T. crassum* from five provinces in Thailand i.e. Ubonratchathani, Sakonnakorn, Mahasarakham, Srisaket, and Nakornratchasima were analysed for isozyme variablility on polyacrylamide gel electrophoresis with 11 enzymes systems : isocitrate dehydrogenase, leucine aminopeptidase, acid phosphatase, phosphogluconate dehydrogenase, alkaline phosphatase, alcohol dehydrogenase, glucose – 6 – phosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, laccase and esterase. Eight of the systems showed polymorphism. Cluster analysis based on isozyme variability using the NTSYSpc 2.00 and UPGMA methods revealed two clusters at a similarity coefficient of 0.67. The first cluster consisted of monokaryotic isolates from Nakornratchasima, Mahasalakham, and 8 samples of Ubonratchathani. The second cluster consisted of the isolates from Sakonnakhorn, Srisaket and 30 samples of Ubonratchathani.
The genetic variations of 9 additional samples of *T. crassum* from four additional provinces i.e. Roiyed, Burerum, Patumthani and Nakhon Pathom were studied by the technique of PCR - RFLP. Two pairs of primers, ITS1-ITS4 and O1-LR12R were used respectively for PCR amplification on ITS (internal transcribed spacer) and IGS (intergenic spacer) regions of the nuclear ribosomal gene, following by digesting with *Hind*III, *Dde*I, *Hae*III, *Eco*RI and *Hinf*I. Data analyses of the PCR - RFLP products based on the similarity index and the UPGMA method in WinBoot program revealed three clusters which were related to their geographic origins except the samples from Burerum which showed genetic variation from the same areas at the similarity coefficient of 0.8 and were grouped into the third cluster.

PS8-456-0235

Geographic variation in the genetic structure of Australian populations of Melampsora lini: implications for regional coevolutionary dynamics.

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Coevolutionary processes occur within heterogenous environments and encompass a range of different spatial scales. Here we use a range of molecular, experimental and field approaches to demonstrate how broad scale patterns of environmental heterogeneity, phylogenetic divergence and regional adaptation influence coevolutionary processes in the *Linum-marginale-Melampsora lini* plant pathogen interaction. Using pathogen isolates collected from 35 locations across southern Australia, we show that there are two broadly distributed lineages of *Melampsora lini* in Australia, and reveal a potential hybrid origin of one of the lineages. In addition, we demonstrate marked variation between these lineages in terms of geographic distribution, pathogenicity and life-history characteristics. We also report on geographic variation in disease epidemiology and host resistance structure for two regions dominated by each of the respective pathogen lineages. These results highlight the importance of integrating molecular, ecological and epidemiological approaches to studies of coevolution.

PS8-457-0264

Phylogeographical analysis of some species in the Umbilicariaceae based on the nrDNA ITS and partial LSU sequences

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One of the intriguing and understudied questions in the biogeography of macrofungi and macrolichens is the relationships between putative eastern Asian and eastern North American disjunct species. Results from the studies on the boreal disjunct distributions of some macrolichens demonstrate that the disjunct distribution of macrolichens is usually only seen at species or lower taxonomic levels. The results, however, are greatly influenced by authors' species concepts and interpretations of morphological characters. Thus, the phylogeny analysis of putative disjunct lichen species is necessary to rigorously test the eastern Asian-eastern North American affinity hypothesis. Here we examine relationships of four species within two genera, *Lasallia* and *Umbilicaria* chosen as examples of disjunct and vicarious distribution patterns. Nuclear ribosomal DNA ITS and partial LSU sequences data were used to construct phylognies for such species. Sequences were aligned with DNAMAN4.0 and transferred to PAUP* 4.0b8a. The most parsimony tree was obtained using the heuristic search with TBR branch swapping and up to 1000 random–addition sequence replications. Clade stability of most parsimonious trees was estimated by 1000 bootstrap replicates using the same parameter set up in the initial heuristic search. MEGA version 2.1 was applied to construct the neighbor-joining (NJ) trees for different data sets according to Kimura's two-parameter model. The genetic distance was also calculated with MEGA based on number of nucleotide difference, positions with gaps and missing data were excluded. All of three tested putative disjunct species pairs were supported. *U. muehlenbergii* and *L. pennsylvanica* are conspecific disjuncts, while *U. esculenta* from China and *U. mammulata* from eastern North America are allospecific disjunct. Morphological similar material of *U. esculenta* and *U. mammulata* formed a monophyletic group, which is sister to the clade formed by *U. muehlenbergii* and *U. subglabra*. These two species share a most recent common ancestor. The ancestor may occur in some areas near the Arctic region and Bering Strait. After the isolation of Eurasian North American continents, divergence following geographical isolation from a common ancestor followed two different paths, with changing in morphology and ITS sequence. In Eurasia, it dispersed along mountains in eastern Asia to south, and in North America, it spread to south along the Appalachia Mountain. Genetic difference between geographic isolated populations of species in Umbilicariaceae from China and North America can be demonstrated by using sequence data of ITS region in the nuclear ribosome RNA gene, and supported by the data of partial LSU region.

PS8-458-0267

Population Biology Of The Sapstain Fungus, *Ophiostoma* ips, Reflects Global Movement Of Its Bark Beetle Vectors

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Bark beetles commonly infest conifers and live in a close association with fungi, especially *Ophiostoma* species and their anamorphs. *Ophiostoma ips* is a common fungal associate of various bark beetle species in their native ranges and has been introduced into non-native pine plantations in the Southern Hemisphere. In this study, we consider the population biology of *O. ips* in native and non-native areas to characterize host specificity, reproductive behavior, the potential origin, and spread patterns of this fungus, together with its insect vectors. Ten pairs of Single Short Repeat (SSR) markers were used to examine the structure of seven populations of *O. ips* including four native populations from Cuba, France, Morocco and USA, and three introduced populations from Australia, Chile and South Africa. The SSR markers across 10 loci examined resolved a total of 41 alleles and 93 genotypes across all populations. Higher genetic diversity was found in the native populations than in the introduced populations. Most alleles were present in all native populations although allele frequencies among populations varied. There was no evidence of specificity of the fungus to particular bark beetle vectors and hosts. Although *O. ips* is homothallic, recombination occurred in the four native populations surveyed. Genetic relatedness of the fungal isolates both from native and exotic environments confirmed the origins of the fungus and its insect vectors. Most alleles observed in the native European population were also found in the native North American population, and this could be due to multiple introductions of European vectors to North America. The higher genetic diversity in the North American population than in the European population suggests that North America would be the most probable source region of *O. ips.*

PS8-459-0275

Phylogeography, cryptic speciation and hybridization in the cellar fungus *Coniophora puteana*

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Fungi often have complex and unpredictable population structures and phylogeographic patterns due to their highly variable life history characteristics. The occurrence of unknown biological mating barriers adds an extra layer of complexity to the analysis of genetic variation in fungi. In this study, we explored the genetic variation and phylogeographic structure in a global sample of the cellar fungus *Coniophora putana*, which is an important destroyer of wooden constructions indoor. DNA sequences were obtained from three independent nuclear DNA loci (beta tubulin, nrDNA ITS and translation elongation factor). The genealogies revealed the occurrence of three separate lineages in the morphotaxon *C. puteana*, apparently representing three cryptic species (PS1-PS3). One of the lineages (PS3) seems to be restricted to North America while the other two have wider distributions, occurring on different continents. In these two lineages (PS1 and PS2), there was little correspondence between genetic and geographic separation, apparently reflecting high gene flow at intercontinental scales. Our data demonstrate that the three lineages reproduce mainly by outcrossing. All three lineages occur in sympatry in North America and our data indicate that hybridization and subsequent intralocus recombination, leading to mosaic sequences, have occurred among two of the lineages in this region (PS1 and PS3). We hypothesize that these two lineages evolved in allopatry earlier, succeeded by a more recent reoccurrence in sympatry, enabling reticulate evolution. Thus, biological barriers to gene flow have apparently not yet evolved between two of the lineages. This study supports the view that cryptic speciation is a very common phenomenon in fungi.

PS8-460-0276

Serpula lacrymans as a model species to study micro-evolutionary processes

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In this project, we use the dry rot fungus *Serpula lacrymans* as a model species to investigate topics within the fields of fungal phylogeography, population genetics and evolution.
Serpula lacrymans causes damages in buildings in temperate regions worldwide and has been found in natural forest environments as well. *S. lacrymans* includes two varieties; one mainly occurring in forests in Northern California (var. *shastensis*) and one variety including isolates in buildings and more exceptional in nature from all continents (var. *lacrymans*). Although the two varieties are able to mate in culture, we obtained support that they act as two biological species in nature.
Data obtained through microsatellite analyses, AFLP and DNA sequencing indicate that var. *lacrymans* has a wide natural distribution in North East Asia and that this region could represent the source population from where the fungus invaded human-made habitats. It seems as the fungus has colonized the human domain independently in Asia and Europe. The extreme genetic homogeneity in the indoor European population indicates that this population established through a recent founder event. Long distance dispersal events have happened to North and South America and Oceania, most likely from Europe.
Very few vegetative incompatibility (*vic*) alleles have been transmitted out from the source population, entailing that genets from the indoor population of *S. lacrymans* in Europe often cannot recognize self from non-self due to shared *vic* genotypes. Identical *vic* genotypes are found in Europe, North America and Oceania, reflecting recent transcontinental dispersal events to these regions. We found little evidence for strong frequency dependent selection being in act on the *vic* loci, as has been hypothesized.
Although little genetic variation was detected with microsatellites and AFLPs in the indoor population of *S. lacrymans*, high levels of sequence variation was found in a gene (MIP), previously shown to be linked to one of the two mating (MAT) loci. This observation indicates that strong frequency dependent selection is acting on the MAT alleles in the indoor *S. lacrymans* population.

PS8-461-0288

Intraespecific variability assessment in *Clavulina coralloides* complex, inferred from ITS region and morphological data

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Clavulina J. Schröt (Cantharellales, Basidiomycota) is a widespread genus, characterized by its clavariod basidiomata, and microscopically, by its basidia with stichic nuclear division (Maire 1902, Donk 1933), often 2-spored and sometimes septate (Corner 1950). The stichic nuclear division is shared with *Cantharellaceae* and *Hydnaceae*, forming the Cantharelloid clade (Pine *et al.* 1999; Hibbett & Thorn 2001).
Despite the genus having about 32 species in the world (Kirk *et al.* 2001), only 3 or 4 species are considered to occur in Europe (Maas Gesteranus 1976, Breitenbach & Kranzlin 1986, Corfixen *et al.* 1997), namely *C. amethystina* (Bull.: Fr.) Donk, *C. cinerea* (Bull.: Fr.) J. Schröt., *C. coralloides* (L.: Fr.) J. Schröt. (= *C. cristata*), *C. rugosa* (Bull.: Fr.) J. Schröt. on the basis of the colour, branching and presence or absence of cristate apexes. Such characters are, however, variable, the reason which lead Corner (1950) to accept 23 form and varieties in this group. Indeed, basidiomata differing in the cited characters can be often found growing from an apparently single mycelium. Furthermore, the parasitic fungus species *Helmintosphaeria clavariarum* (Desm.) Fuckel can cause a change in some of the cited features, increasing the variability. Martin *et al.* (2003) suggested that *C. rugosa* could have an abnormally developed form, infected by phytoplasms, as proved in *Ramaria*. Thus, the identification of the European species being difficult, the aim of this work is to assess the enormous variability of the *C. coralloides* complex, in order to provide a better understanding of the taxonomy of the group. Molecular and morphological data have been used.
About 250 European collections of *Clavulina* kept in the following Herbaria, ARAN-Fungi, BC, BCC, BIO-Fungi, GDAC, LISU, LOU-Fungi, MA-Fungi, and UPS, were examined.
The amplification of ITS region of 25 collections was carried out, using ITS1, ITS1F, ITS4 and ITS4B primers (White *et al.* 1990, Gardes & Bruns 1993). Purified PCR products were directly sequenced in both directions on an ABI 310 DNA Sequence Analyzer (Applied Biosystems) automated sequencer, following the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction DNA Sequencing Kit protocol. The forward and reverse sequence readings for each DNA sample were assembled using the BIO EDIT version 5.0.9 program. A number of sequences were retried from the GenBank.
Phylogenetic analyses on the resulting ITS alignment were conducted with the software PAUP.

PS8-462-0346

Geographical distribution of intraspecific groups of Umbelopsis ramanniana and the genetic variations of nLSU rDNA in their local populations

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Umbelopsis ramanniana (A. Möller) W. Gams is one of the most ubiquitous species in this genus and its sporangiospore shape is remarkably variable. The worldwide distribution and divergent shapes of the sporangiospore of this species imply some intraspecific groups with the genetic variations exist. We have showed that the isolates of *U. ramanniana* collected in Europe split into at least three intraspecific groups based on sequences of nr DNA ITS regions (Ogawa et al. 2005). However, we could not make clear the geographical distribution of these intraspecific groups because of a small number of the examined strains. In the present study, we will discuss the geographical distribution of the intraspecific groups of *U. ramanniana* and their genetic variations of nLSU rDNA in their local populations.
Thirty-six strains of *U. ramanniana* were isolated from leaf litter of confers (*Pinus* and *Picae*) collected in Europe, and northern, central, and southern Japan. The single-sporangiospore or single-sporangium isolates grown at room temperature on Miura agar medium were used for the experiments. The nLSU rDNA including D1/D2 regions of these strains were amplified by using HotStarTaq Master Mix (Qiagen) and a primer set of NL1 and NL4 designed for nLSU rDNA (O'Donnell 1993). After purifying PCR products, the DNA sequences were determined directly with the dye terminator method. The variations of the sequences among strains were analyzed using neighbour-joining analysis.
The neighbour-joining analysis showed that the examined strains split into three intraspecific groups with some subgroups although the bootstrap values were not so high. Group I comprised 6 subgroups, Group II 2 subgroups, and Group III 5 subgroups. Interestingly, most of subgroups consisted of the strains isolated from the same sampling area. For example, of 6 subgroups in Group I, one subgroup included only the strains isolated in southern Japan, the two subgroups only the strains in Europe, and other three groups only the strains in northern Japan. Furthermore, in some cases, the shape of the sporangiospore differed subtly among subgroups. These facts suggest that the local populations of *U. ramanniana* with genetical variations distribute corresponding to environments.

PS8-464-0384

Genetic variation in the SSU intron and the dispersal and migration history in Sweden of Cliostomum corrugatum.

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The aim with this study is to determine genetic variation, dispersal potential and the migration history to Sweden since the last glaciation of the rare lichen *Cliostomum corrugatum*, a crustose epiphytic lichen with a grey greenish thallus, conspicuous light yellow to light brown apothecia and black pycnidia. Collections were made in January and February in 2005 at five sites in Östergötland, Sweden. The most frequent common habitat for *Cliostomum corrugatum* is on *Quercus* and sometimes also on other deciduous trees for example *Ulmus* and *Fraxinus*. On the tree trunk it is the rough bark it prefers and the flat terminal parts of the bark structure and not on the sides of the cracks. The main distribution of *Cliostomum corrugatum* is in Europe but a satellite population has been found on the west coast of North American in British Columbia. It is red listed in Sweden, with the status near threatened.Three sequences SSU intron, IGS and ITS were studied and the two latter appear to lack genetic variation. A total of 85 sequences with a length of 614 base pairs were studied of a rRNA SSU intron. Eleven haplotypes were detected, two was common 46 and 30 in numbers respectively and was present on all five localities the other nine were detected only once each. The two common haplotypes are in the centre of a rooted net work and the rare in the periphery. *Cliostomum corrugatum* does not seem to have problem with its dispersal. The limiting factor seems to be the occurrence big oaks. In the studied area the smallest tree trunk diameter that *Cliostomum corrugatum* was found on is 0,65 metre. The tree with the largest diameter in the research area is 1,65 metre. A tree that is 0,65 metre in diameter is at least 100 years old. Oaks of this age are scarce and this is one of the reasons for the rareness of *Cliostomum corrugatum*. When *Cliostomum corrugatum* colonized Sweden after the last ice age, all eleven haplotypes may already have existed. However, it is possible, that some haplotypes evolved after the migration to Östergötland.

PS8-465-0394

Use of 18S rDNA and TGGE to study aquatic hyphomycetes in polluted surface and groundwater

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Aquatic hyphomycetes initiate the degradation of organic material in rivers and ponds and are therefore efficient contributors to the food web of aquatic ecosystems. Surprisingly, the litter is also colonised and degraded in aquatic habitats contaminated by heavy metals or organic xenobiotic compounds. The ecology and biodiversity of aquatic fungi is therefore of considerable interest and hence the composition and the dynamics of natural fungal communities in polluted environments require further investigation.
We studied communities of aquatic hyphomycetes on alder leaves exposed in three surface water and two groundwater habitats representing a pollution gradient due to sulphate, nitrate and heavy metals. Communities were characterized with molecular and morphology-based methods. The genetic marker NS1/GC-fung was used for nested PCR amplification followed by Temperature Gradient Gel Electrophoresis (TGGE). Generally, the molecular approach revealed fewer phylotypes in surface waters than the morphology-based method. The opposite was found in groundwater. Extremely low numbers of conidia and ergosterol concentrations were found in groundwater and in the highly polluted surface water habitat H4. In the latter, after two weeks of leaf immersion only 1.6 conidia and 0.04 "micro" g ergosterol per mg dry leaf mass, as opposed to 927 conidia and 0.11 "micro" g ergosterol per mg dry leaf mass in the moderately polluted surface water habitats H8 and H9 were observed.
Our data based on a methodologically combined approach show that harsh ecological conditions might delay substantially the ecological key process of fungal leaf decomposition by changing fungal biodiversity.

PS8-466-0403

Congruence found among recombination rates and population ages for different populations of *Mycosphaerella graminicola*

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I wanted to test the simple hypothesis: that more recombination events are likely to be fixed in an older population than in a younger population assuming similar population structure and environmental condition. Intragenic recombination rates were estimated for 5 worldwide populations using 6 DNA sequence loci, in which intragenic recombination was observed. We used information about population structure from earlier studies done on *M. graminicola* to determine the age and demographic history of the five populations. The findings mostly support the hypothesis of recombination rate reflecting population age. One local population of a relatively young age showed a high recombination rate. High levels of directional gene flow from the ancestral population can be responsible for carrying on the signal of recombination to newer populations in some cases.

PS8-467-0414

Population Genetic Studies Of Symbionts In Lichens With Different Propagation Modes

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Symbiotic associations of fungi with algae are regarded as an important key innovation in the evolutionary radiation of ascomycetes. However, there is still a gap of knowledge concerning the symbiont selectivity patterns, especially at the intraspecific scale.
In our population genetic study we focus on two species of *Physconia* with a comparatively high symbiont selectivity and different propagation strategy. One species (*P. distorta*) is generally producing ascospores that sample algae from a "pool" in the environment, while the other species (*P. grisea*) propagates both symbionts jointly by special structures, the soredia. In both cases we observe a wide distribution of some haplotypes of lichen symbionts.
By comparing populations of the mycobiont and the associated photobiont we find diverse patterns between the studied lichen species with different propagation modes. We show that the intraspecific variation of the symbionts of the sexually reproducing lichen is similar across various altitudinal levels, whereas the highest gene diversity of the asexually reproducing lichen is found between 200-400m. The photobiont diversity is comparable in both species and photobiont switches between them seem to be frequent. The vegetatively reproducing mycobiont however shows the lowest diversity of all investigated symbionts. We will compare these patterns also with findings in other lichen species, and address the question whether photobiont selectivity could play a role in speciation.

PS8-468-0434

Evolution of microsatellites in the mitochondrial genome of *Rhynchosporium secalis*

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The aim of this project was to investigate the evolution of microsatellites in a mitochondrial genome, more specific to test hypothesis that the microsatellites evolve faster than the variable nucleotide regions and therefore could add to the diversity of the genome.
Two variable microsatellite regions in the genome of *Rhynchosporium secalis* were identified. The microsatellite repeats consisted of 2 and 7 base-pair repeat respectively. For the two loci, four and three alleles were identified among 60 worldwide isolates of *R. secalis*. Variable flanking regions of the microsatellite and other variable regions of the mitochondrial genome were sequenced in order to infer a phylogeny representing the mitochondrial genome. No phylogenetic conflict was observed among the regions sequenced. All the sequence loci were therefore treated as one DNA sequence locus. A haplotype network was generated from the combined sequence data and a total of 11 haplotypes was found. Only one missing haplotype was identified in the network, suggesting a good representation of the diversity found in the mitochondrial genome. After mapping the microsatellites onto the haplotype network, we found that one microsatellite increased the diversity in the haplotype network. The other microsatellite was responsible for a conflict by introducing homoplasy in the network, suggesting that the same microsatellite repeat number might have arisen twice. We conclude that the microsatellites found in the mitochondrial genome of R. secalis will be useful to analyse populations of *R. secalis*. It also suggests that the mutation rates for the microsatellites are higher than the nucleotide substitution rates in the mitochondrial genome.

PS8-469-0439

Population expansion-migration scenarios explain the demographic history of the fungal pathogen *Mycosphaerella graminicola*

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The following hypothesis was tested in this project: a population that evolve with a new host is likely to show high levels of population expansion over time, where as population recently founded by gene flow show no population expansion and a more disruptive phylogenetic network pattern.
Five global populations of *Mycosphaerella graminicola* were chosen for this study and information from 6 DNA sequence loci and four microsatellite loci was used to estimate the different population genetic parameters and infer haplotype networks. Haplotype networks from recently founded populations showed more disruptive networks, where as the populations that potentially had evolved with the new host showed star like networks. Two different models were used to estimate mainly population expansion and migration, the first model allowed for recombination and estimated directional gene flow as well as possible growth for each population. The second model did not allow for any recombination, but estimated divergence time, migration and population expansion between two populations at the time. Both models supported the same scenarios where significant population expansion where seen in the populations evolving with the host and no expansion in the recently founded populations. The migration estimates helped to explain this pattern as most migration where coming from the populations evolving with the host to the recently founded populations. We conclude that population expansion might play an important role in shaping the population structure of a pathogen evolving on a new host, given that the new host is successful

PS8-470-0444

Origin And Expansion Of *Rhynchosporium Secalis,* A Fungal Leaf Pathogen Of Barley

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Rhynchosporium secalis is a Deuteromycetous fungus with a proposed teleomorph in the Discomycetes. However, the teleomorph has never been found and its existence is solely based on observed moderate levels of neutral genetic diversity and frequency-dependent-selection on mating types as measured in some populations. It is a pathogen that infects cultivated barley and other *Hordeum* relatives as well as eg couch grass (*Agropyron repens*), a common grass species native to Eurasia. Because of its common association with cultivated barley, a coevolutionary relationship between *Hordeum* species and *R. secalis* is assumed, which suggest a Middle Eastern origin for *R. secalis*. However, neutral genetic variation in *R. secalis,* as obtained with RFLPs and 15 microsatellite loci, consistently reveal a significant lower number of alleles and lower levels of gene and genotypic diversity in *R. secalis* populations originating from cultivated barley in the Middle East, as well as from the non-cultivated *Hordeum spontaneum* populations from the Middle East, the progenitor of cultivated barley. This suggests that *R. secalis* did not co-evolve with barley, but co-evolved with another host and only encountered barley when it was introduced as an agricultural crop.

PS8-471-0509

Genotypic diversity of *Armillaria fuscipes* in South African Pine plantations

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Armillaria fuscipes (Basidiomycetes, Agaricales, Tricholomataceae) is the causal agent of *Armillaria* root rot on various economically important Pinus species planted in South Africa. The taxonomy of this fungus has been well established in the country but its population structure in pine plantations has not been considered. The aim of this study was to ascertain the genetic diversity of *A. fuscipes in P. elliottii* and *P. patula* plantations of South Africa. Isolates were collected from plantations in the Mpumalanga and Limpopo provinces. RFLP analyses of the IGS-1 region were performed on the fungal isolates to confirm their identity. The genotypes of the isolates were assessed using vegetative incompatibility tests. These tests were conducted by crossing all the isolates with one another in all possible combinations on malt extract agar. Genetically identical isolates were identified by the absence of a demarcation line between the crossed isolates. AFLP analyses were employed to further assess the genetic diversity of the isolates. Banding patterns for these analyses were obtained using a variety of primer sets. RFLP profiles typical of *A. fuscipes* were obtained for all isolates. Isolates collected within discrete infection centres were found to represent single clones of *A. fuscipes*. Those from different centres were typically of different genotypes. Preliminary AFLP analysis yielded a low number of polymorphic bands, indicating that the genetic diversity among the isolates is very low. The current view, based on the small number of genotypes observed in this study, is that *A. fuscipes* spreads by means of vegetative growth or transfer of mycelium on infected wood, within infection centres. This knowledge should contribute to the management of this pathogen in South African pine plantations.

PS8-472-0520

Genetic diversity of *Fusarium thapsinum i*solates from different hosts in Australia

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Recent studies indicate that *Fusarium thapsinum* is associated with grain sorghum, grass weeds and native grasses in Australia. The presence of *F. thapsinum* in grasses in non-agricultural ecosystems such as grasslands and woodlands could be due to the coevolution of the fungus with some native grasses or its spread from one system to another.
We tested the hypothesis that the genetic pattern of *F. thapsinum* isolates is correlated to host, and agricultural/non-agricultural ecosystem.
Seventy-one isolates of *F. thapsinum* were recovered from grain sorghum, grass weeds and native grasses in New South Wales or far north Queensland, Australia (Table 1).
Male/female fertility of each *F. thapsinum* isolate was determined by sexual compatibility tests with tester strains of *Gibberella thapsina* (teleomorph). Genetic patterns were based on AFLP analysis with three primer pairs, *Eco*RI-GG/*Mse*I-CT, *Eco*RI-AA/*Mse*I-AT and *Eco*RI-TG/*Mse*I-TT.
All isolates of *F. thapsinum* were male fertile but three isolates were also female fertile (Table 1).

Table 1 Mating types and male/female ratios of *F. thapsinum* isolates from different hosts and locations

Host		Location	Ratio *MAT-1 : MAT-2*	Ratio Male : Female	Total
Agricultural ecosystem					
Sorghum bicolor	Grain sorghum	NSW	12 : 19	30 : 1	31
Sorghum halepense	Grass weed	NSW	14 : 6	19 : 1	20
Echinochloa crus-galli	Grass weed	NSW	1 : 0	1 : 0	1
Non-Agricultural ecosystem					
Austrostipa aristiglumis	Native grass	NSW	1 : 5	6 : 0	6
Coix gasteenii	Native grass	QLD	6 : 3	8 : 1	9
Heteropogon triticeus	Native grass	QLD	0 : 1	1 : 0	1
Sorghum interjectum	Native grass	QLD	0 : 3	3 : 0	3
Total			**34 : 37**	**68 : 3**	**71**

AFLP analysis generated 138 markers, of which 97 were polymorphic. All isolates of *F. thapsinum* were clustered together within the range of 81-99% based on the UPGMA cluster analysis on the basis of DICE coefficient.
In general, isolates did not cluster according to their hosts or ecosystems. Nevertheless, the highest genotypic similarity was among isolates of *F. thapsinum* from the same grain sorghum plant (≥96%) or grain sorghum plants from one location (≥97%).
Isolates of *F. thapsinum* from grain sorghum, grass weeds and native grasses showed similar biological traits (Table 1) and were not genetically clustered separately according to the ecosystem. Sexual compatibility of isolates as males and females indicated that there is no reproductive isolation among isolates from different hosts or ecosystems.
Isolates from grain sorghum and grass weeds in agricultural ecosystems were shown to be genetically similar to those from native grasses in non-agricultural ecosystems, with relatively high genetic similarity of 81%.
Therefore, the hypothesis that the genetic pattern of *F. thapsinum* isolates is correlated to host, and agricultural/non-agricultural ecosystem is rejected.
Ria Johansen, Hien Phan Tien, Jillian Walsh and Shireen Quazi kindly provided isolates of *F. thapsinum* from grasses. The senior author acknowledges the support of the Thomas Lawrence Pawlett Postgraduate Scholarship.

PS8-473-0526

Genetic diversity and substrate preferences in *Hypogymnia physodes*, in northern Europe

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Genetic variation in lichens has mainly been examined in rare or threatened species or species with an otherwise fragmented geographical distribution. The main objectives have often been to compare the diversity between populations in relation to nature conservation issues. In addition, most studied species are sexual reproductive and, hence, produce small spores which may disperse over long distances. More common species have usually been neglected, although they are more easily collected, both because collecting results in a comparatively small disturbance of the populations and because they occur in a larger selection of habitats. Here we present a study on the genetic variation in the lichenized ascomycete *Hypogymnia physodes* in Northern Europe based on nrDNA data. The species was selected as it probably is the most common lichen in the area, it is corticolous, found on almost all woody plants in most habitats, and has a predominantly asexual dispersal mode. The material was collected in Estonia, Finland, and Sweden as a part of a larger project aiming at identifying localities with high biodiversity of interest for nature conservation projects.
We examined the correlations between genetic diversity and substrate ecology as well as spatial distances.
An important result is the large genetic variation within a mainly asexual lichen species. The results also show genetic similarity between specimens from similar substrates.

PS8-474-0528

Patterns of genetic diversity of *Pseudevernia furfuracea* compared with chemistry, morphology and substrate ecology

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Common lichen species have usually been neglected in studies of genetic variation, although collecting of these results in a comparatively small disturbance of the populations, they occur in a larger variety of habitats, and thus, are more easily collected. Instead genetic variation in lichens has mainly been examined in rare or threatened species or species with a fragmented geographical distribution. This is reasonable as the main objectives usually have been to examine the diversity between populations in relation to nature conservation issues. In addition, most studied species are sexual reproductive and, hence, produce small spores which may disperse over long distances. Studies of asexually reproducing and common lichens are rare. Based on nrDNA sequence data this study aims at describing the genetic variation in the lichenized ascomycete *Pseudevernia furfuracea* in Northern Europe and to compare this variation with morphology, secondary metabolite chemistry, and substrate ecology.
This isidiate species is one of the most common lichens in the area, it is corticolous, found on almost all woody plants in most habitats, and has a predominantly asexual dispersal mode. Apothecia are very rare, less than 1 % of the herbarium material is labelled as fertile. The specimens for the study were collected in Northern Europe as a part of a larger project aiming at identifying localities with high biodiversity of interest for nature conservation projects.
The genetic variation within this mainly asexual lichen is compared with variation in morphology, secondary metabolite chemistry, and substrate ecology.

PS8-475-0551

Development of microsatellite markers to study the population biology of the wood-inhabiting fungus, *O. quercus*

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The *Ophiostoma piceae* complex encompasses a suite of taxa that inhabit the wood of conifers and angiosperms. They are generally known as sap stain fungi and have a worldwide distribution but are thought to be native to the Northern Hemisphere. Well known species in the complex are *O. piceae* and *O. quercus* that are morphologically similar and were treated as a single taxon for many years. They are now known to typically occur on either hardwoods (*O. quercus*) or conifers (*O. piceae*). Phylogenetic studies have shown that these two species can be separated based on DNA sequence comparisons as well as on mating compatibility. It has been suggested that *Ophiostoma. quercus* is native to Europe and was introduced into the Southern Hemisphere and North America. However, previous studies have found that *O. quercus* is extensively distributed throughout South Africa on native and exotic hardwoods. In addition it has been reported from other Southern Hemisphere countries. The extensive distribution of *O. quercus* in the Southern Hemisphere has resulted in uncertainty concerning its centre of origin. The aim of this study was to develop microsatellite markers that can be used to consider the population biology of *O. quercus* isolates from hardwood trees from various parts of the world. Polymorphic microsatellite markers were developed using an enrichment protocol, FIASCO. Sequences obtained from this enrichment revealed 22 microsatellites including a mixture of di, tri, tetra and hexanucleotide repeats. Primers were designed flanking these microsatellites and tested for polymorphisms. The polymorphic primers will be used in population genetic studies to provide new information on the genetic diversity and movement across populations of *O. quercus*.

PS8-476-0640

Phylogeographic patterns of *Armillaria ostoyae* in the western United States

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Throughout its circumboreal distribution, *Armillaria ostoyae* is a principle cause of Armillaria root disease of conifers. While *A. ostoyae* is generally thought of as highly pathogenic, it can exhibit diverse pathogenicity, virulence, and ecological behavior. Also, previous research has noted distinct differences in *A. ostoyae* epidemiology among coastal and interior regions of western North America. Although genetic variability has been observed within *A. ostoyae*, this variation has not been studied in depth. The aim of this study was to identify genetic differences among diploid genets of *A. ostoyae* from the western USA, and examine intraspecific and interspecific phylogeographic relationships based on gene trees derived from nuclear ribosomal DNA (rDNA). A total of 77 genets of *A. ostoyae* (73 genets from western USA and one genet each from Mexico, Finland, Russia, and eastern USA, respectively) were examined in this study. Direct-PCR was used to obtain sequences of rDNA regions (i.e., large subunit, internal transcribed spacer, 5.8S, and intergenic spacer) from each *Armillaria* genet, and extra procedures were used to decipher many heterogeneous rDNA-sequence types. Sequence analysis using Bayesian inference methods defined three phylogenetic groups. Two phylogeographic groups were associated with the Rocky Mountain and Pacific Northwest regions of the USA. Additional analysis of *A. ostoyae* from outside the western USA indicates the presence of a circumboreal group with representation in Utah, USA. Individual genets containing heterogeneous rDNA-sequence types from multiple groups were common in some geographic regions. Intragenomic variation in rDNA-sequence types is perhaps derived from common evolutionary ancestry and/or intraspecific hybridization. Hypothetically, groups may have physically converged after long-term geographic isolation. Subsequent hybridization events may have influenced evolution and contributed to variation in ecological behavior of *Armillaria* species.

PS8-477-0662

Investigating the genetic diversity of *Puccinia boroniae* in Western Australia.

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Puccinia boroniae is an economically important pathogen in commercial *Boronia* plantations in Western Australia. To address the question of whether one or more species of *P. boroniae* are present, an initial study was conducted. Genetic diversity among field specimens collected from commercial *Boronia* plantations was assessed by analysis of the polymorphism within the intergenic spacer 2 (IGS2) region of the nuclear ribosomal RNA genes.

The IGS2 region of 22 *P. boroniae* specimens was amplified with primers CNS1 and NP. PCR products were subsequently digested with a selection of restriction enzymes and analysed by gel electrophoresis. Five representative samples were purified, cloned into pCII-TOPO vector, the cloned fragments sequenced and aligned.

Two different RFLP profiles were generated. Most specimens, sampled from a range of *Boronia* species and collected from plantations at different geographical locations, produced a homologous RFLP profile (Group 1). Three specimens (Group 2), obtained over three sampling periods from *Boronia megastigma* at a single plantation, produced a different profile. A single specimen also collected from this same plantation, but isolated from *B. heterophylla* belonged to Group 1.

Comparison of sequence data generated from representative specimens from each profile group showed that single point mutations at endonuclease recognition sites were responsible for the changes in profile. Sequence alignment also highlighted several insertion/deletion events common to the Group 2 specimens.

Variation between single telia of *P. boroniae* collected from individual *B. megastigma* plants at the plantation exhibiting the Group 2 profile was examined by PCR-RFLP analysis of the nuclear internal transcribed spacer (ITS) region. A single homologous profile was observed.

Overall, no segregation of *P. boroniae* according to host specificity was concluded, though the data suggested the possible presence of a subspecies (race) of *P. boroniae,* that is isolated geographically and possibly host (cultivar) specific. Further investigations involving pathogenicity trials are required. The low level of diversity observed in this study may also be a reflection of the influence of human distribution on the pathogen, as the rust is rarely observed in wild populations.

An interesting aspect of the study was the presence of a new spore stage (pycnial stage) recorded only at the plantation at which the Group 2 specimens were sampled from. The lifecycle role of this spore stage is unconfirmed at present.

PS8-478-0880

Diversity of *Gibberella zeae* populations isolated from wheat in Argentina

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Argentina produces ~ 16 millon tons of wheat. The main pathogen associated with Fusarium Head Blight (FHB) in wheat in Argentina is *Gibberella zeae* (anamorph *Fusarium graminearum*). Over the last 50 years, epidemics of varying degrees of severity occurred on wheat in Argentina. The objectives of this study were to evaluate the genetic diversity of *G. zeae* populations isolated from wheat in Argentina by Amplified Fragment Length Polymorphism (AFLP). We collected wheat spikes during a severe FHB epidemic (harvest season 2001/02) from various wheat production areas in Argentina. *Gibberella zeae* was isolated from 50 spikes with FHB symptoms from each of several fields planted to a wheat line (Pro Inta Granar) that is highly susceptible to scab. The disease severity was estimated as > 70% at all locations. All of the *G. zeae* strains identified by morphology formed perithecia homothallically on carrot agar. We used three specific primer pair combinations *Eco*+AA/*Mse*+AT, *Eco*+CC/*Mse*+CG, and *Eco*+TG/*Mse*+TT in an AFLP analysis to resolve 216 loci from130 isolates. Of the 216 loci scored, 200 were polymorphic. Populations of *G. zeae* causing FHB on wheat from Argentina include isolates that cluster with phylogenetic lineage 7. The high level of genetic diversity, as measured by AFLP, is consistent with the high level of VCG diversity previously reported from these same populations.

PS8-479-0896

Differences in conjugation patterns and sporidia production between populations of *Microbotryum violaceum var. dioica*

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Mating system is one of the essential factors structuring genetic variation in a species. Consequently mating also influences the conditions for coevolution between species. Mating system traits might also be subject to selection if they are variable and result in fitness differences. Here we studied the pathogenic sterilizing fungus *Microbotryum violaceum,* infecting the host *Silene dioica,* to look for variation among populations in mating system traits. *M. violaceum* is obligately sexual in that the dikaryotic infection hypha that allow the fungus to become systemic in new hosts can only be formed after gametes unite. In meiosis *M. violaceum* produces tetrads in which the cells, two of each mating type, either conjugate or produce sporidial cells that multiply like yeast. Mating can occur between any cell of opposite mating-type, allowing both outcrossing and selfing, but recent studies have shown that automixis in the form of intra-tetrad mating is the predominant mating-system. However, a theoretical model predicts variation between populations in the amount of automixis and a hypothesis has been put forward that involves two different conjugation strategies; intra-tetrad mating giving a time advantage contra production of many sporidia giving a numerical advantage of infectious dikaryons.
By germinating teliospores in two different nutrient levels (mating behaviour in *M. violaceum* has previously been shown to be significantly affected by nutrient level) and studying them under a microscope we asked: Do populations of *M. violaceum* var. *dioica* differ in proportion of automixis, sporidia production and production of infectious dikaryons, and is there any correlation between these traits?
Our results show an interaction between populations and nutrient levels for these traits which might result in altered trajectories for genetic distribution and selection, depending on the conditions under which germination and infection most commonly occur in nature. We also found a negative correlation between rates of automixis and sporidia production, as predicted, but only under high nutrients. However, we also found a strong positive correlation between the extent of automixis and proportion of conjugating sporidia in both nutrient levels. We thus suggest that it is actually not the automixis rate *per se* that are responsible for the reduction in sporidia production, but the overall conjugation propensity. Under high nutrients, this seems to compensate for the negative effect of increased automixis on sporidia production, since our results also show that the ecologically more important production rate of infectious dikaryons is not affected by increased rates of automixis.

PS8-480-0930

Phylogeny And Population Structure Of *Ceratocystis fimbriata* From Different Hosts In Colombia

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Ceratocystis canker caused by *Ceratocystis fimbriata sensu lato* is one of the most destructive diseases of coffee, cocoa and citrus in Colombia. In this study, the pathogenicity, phylogenetic relationships and population structure of *C. fimbriata* isolates obtained from seven different hosts in Colombia, were considered. Phylogenetic analysis based on sequence data for the ITS region of the ribosomal rRNA operon, Mat-2 HMG Box and partial b-tubulin gene, showed that all isolates resided in one of two distinct genetic lineages, previously reported for this fungus in Colombia. These lineages were independent of the host from which isolates were obtained. Pathogenicity tests on coffee, cocoa and citrus plants using isolates from these hosts, showed that the fungi could infect and cause disease on all three hosts inoculated. There was, thus, no indication of host specialization amongst the strains tested. A population biology study using microsatellite markers confirmed the existence of the two distinct lineages for *C. fimbriata* in Colombia. Furthermore, a high population differentiation was shown to exist between these populations. Genetic variability amongst isolates within these lineages was high and linkage disequilibrium analysis suggested a moderate level of sexual out-crossing within each population. Analysis of isolates collected from a single coffee plantation showed that they all belonged to the same genetic lineage. No differences could be detected between isolates originating from soil or symptomatic plants. Knowledge gained from this study, should be useful in designing control strategies against Ceratocystis canker, and especially in breeding programs aimed at developing host resistance to the disease in Colombia.

PS8-481-0931

Genetic and Phenotypic Variability Of *Phytophthora infestans* From Colombia

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Phytophthora infestans is one of the most destructive pathogens of solanaceous crops in Colombia. The aim of this study was to characterize the level of genetic and phenotypic variability of 35 *P. infestans* isolates obtained from several hosts (potato, tomato, tree tomato and pear melon) and regions of this Andean country. Mitochondrial haplotypes (mtHap) were defined using specific primers and those groups were further analysed using RAPD markers, metalaxyl resistance and mating type. Results showed existence of two mtHap (Ia and IIa). The mtHap Ia included isolates obtained on tree tomato from the south of the country, while isolates from potato and tomato, belonged to mtHap IIa. A third mtHap group was found in isolates from pear melon and tomato, but could not be defined based on previous reports. Mating type analysis showed that all the isolates studied belong to the type A1 and a wide range of variation was found with regard to the sensibility of isolates to Metalaxyl, with some isolates growing above 300 mg/L of a.i. RAPD analysis showed a moderated level of variation among isolates from each mtHap (h=0,16), which suggests an important participation of asexual recombination events in life cycle of this oomycete. Interestingly, isolates from tree tomato resided in two related clades, with a DICE similarity index of 0.83. Results from this study suggest that control strategies for late blight of Solanaceous plants in Colombia, should take into account intra-lineage variability, which has been excluded from the population studies of *P. infestans.*

PS8-482-0935

Morphological and genetic methods to differentiate strains of *Phoma clematidina* on Clematis in New Zealand

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A mycological survey of native and exotic *Clematis* (Ranunculaceae) species throughout New Zealand identified *Phoma clematidina* as a relatively common leaf inhabitant and minor pathogen of some species. A pathogenic strain of *P. clematidina* from USA was previously introduced for the biocontrol of *Clematis vitalba,* due to the endemic strains being mildly to non-pathogenic on the target host. Diagnostic techniques to differentiate the endemic strains from the exotic biocontrol strain were explored to help determine which strains were present on the different *Clematis* species surveyed. Isolates were characterised using cultural and morphological characteristics, substrate utilisation and biochemical properties. Genetic methods were also used to characterise the fungus. DNA was extracted from 75 isolates that were selected for sequencing studies. The universal primers ITS1 and 4 were used to amplify ITS 1 and 2 regions of the rDNA. Preliminary *in vitro* inoculation assays were undertaken to determine relative pathogenicity between isolates. Both pathogenic and non-pathogenic strains were identified. Symptoms ranged from asymptomatic colonisation of plant tissues through to necrotic leaf lesions. The survey identified that other endophytic, saprophytic and pathogenic fungi were also associated with *Clematis* in New Zealand and included species of *Alternaria, Colletotrichum, Fusarium, Microsphaeropsis, Phyllosticta* and *Phomopsis*.

1530-1730

SYMPOSIUM 41 - *Evolution, Ecology and Systematics of Endophytic Fungi - Horizontally Transmitted Endophytes*

S41IS1 - 0691

Foliar endophytes versus leaf litter saprobes: annual cycle of an ascomycete community associated with oak leaves

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The competitive strategies employed by fungi associated with living and dead leaves in deciduous forest ecosystems are still poorly understood, and fungal life-cycles in forests need to be studied more closely. Our aims was to determine the species composition of the ascomycete community associated with green leaves and litter of *Quercus robur* (English oak) individuals in a Dutch oak forest, and to investigate the fungal life-cycles involved. Over a period of two years, we isolated endophytes monthly from healthy leaves, monitored fungal sporulation in lesions on attached leaves and on leaf litter. Isolates were identified morphologically and by sequencing the ITS region of the rDNA.
In total 44 ascomycete taxa were found, 34 of which were isolated as endophytes from leaf fragments. On average, 64.5% of fragments were colonized by ascomycetes, with monthly colonization frequencies reaching as high as 100% at the end of each summer. The most frequently isolated taxa were *Mycosphaerella punctiformis* (44%), *Tubakia dryina* (24 %), *Fusicoccum quercus* (6 %), *Naevala minutissima* (6 %), and *Tubakia* sp. (5 %). These taxa accounted for 83% of all endophyte isolates. Of 18 taxa sporulating on fallen leaves in their first year of decay, 10 (56%) were also isolated from green leaves.
The pattern of isolation of the predominant endophyte species *M. punctiformis* indicates that, after infection in spring by ascospores from overwintered leaves, hyphal colonization of the living leaf tissues occurs. The inoperculate discomycetes *Naevala minutissima* and *Brunnipila (Dasyscyphus) fuscescens* also infect leaves via ascospores from apothecia that develop in summer on the overwintered leaves, but their colonization frequencies rise relatively late in the growing season. The high colonization frequencies seen throughout the growing season with *Tubakia dryina* and *Tubakia* sp. can only be explained by an early colonization arising from twigs. Our results indicate that the dominant taxa employ different strategies to compete for the available foliar tissues. Several species that are generally regarded as 'saprobes', show an endophytic phase in their life-cycle. By colonizing the living tissue at an early stage, these 'sapro-endophytes' have an advantage over purely saprobic species, which are able to invade leaves only when the tissues die.

S41IS2 - 0605

Host specificity among endophytes in transient plant communities

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Horizontally transmitted endophytes are ubiquitous hitchhikers on the plants of the world. While the interior of a leaf may seem a stable habitat, populations of host plants themselves come and go. How do populations of host-specific endophytes maintain themselves in the face of vegetational change?
Answers to this question lie in an understanding of endophyte life cycles, host plant succession, and the distributions of both hosts and endophytes. Such information is spottily available for just a few groups of endophytes, including *Rhabdocline parkeri* on Douglas fir, Xylariaceous endophytes on a huge variety of hosts, and species of *Phyllosticta*.
Rhabdocline parkeri occurs only on Douglas fir, but successful infection depends strongly on genotype compatibility between host and endophyte. Genotypic diversity of *Rhabdocline* in very young host stands is low. However, within 15 years that diversity has increased by orders of magnitude, presumably as a result of migration and sexual reproduction by the endophyte.
Xylariaceous endophytes apparently show no host specificity. For example, *Nemania serpens* occurs infrequently in most of the trees and under-story species in an old growth Douglas fir forest in Oregon. However, the sexual phase is found only on *Acer macrophyllum*, an early successional species in a Douglas fir forest. As *Acer macrophyllum* gives way to Douglas fir, dispersed endophyte infections serve to preserve *Nemania* in the habitat until its preferred host becomes available again. Transmission from Douglas fir needles to *Acer* wood occurs, at least under laboratory conditions. The *Nodulisporium* state has never been seen on coniferous needles directly collected from the wild, and it is unknown whether conidia can serve to disperse the endophyte among non-preferred hosts. It is also unknown how long the endophyte can persist in the absence of *Acer mcarophyllum*.
Phyllosticta is one of the quintessential groups of endophytic fungi. The genus is prevalent on conifers, on liliaceous plants, and on evergreen angiosperms worldwide. The group is particularly speciose in semiarid tropical/sub-tropical habitats. Host-specificity ranges from non-existent to highly precise. DNA sequence data reveals that most isolates from moist forests in warm climates are *Phyllostica capitalensis*, whatever the host. This fungus must be the coelomycetous equivalent of *Cladosporium sphaerospermum*. Conversely, isolates from areas of seasonal limited rainfall are likely to prove host specific, but not absolutely so. Long-term vegetational change may leave endophytic *Phyllosticta* strains stranded on non-host plants, a situation likely to foster speciation.

S41IS3 - 0813

Endophytes: lifestyle and phylogenetic diversity

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Fungal endophytes and saprobes generally play an important ecological role within plant tissues, decayed plant material and pine needles. Several reports based solely on morphological observations have postulated that there is an intimate link between endophytes and saprobes. This study aims to provide valuable insight as to whether some endophytic fungi change their ecological strategies and become saprotrophs upon host decay. Ribosomal DNA based sequence analyses from 99 isolates (endophytes, mycelia sterilia and saprobes) recovered from *Magnolia liliifera* suggest there are specific taxa that possibly exist as endophytes and saprobes. Isolates of *Colletotrichum, Fusarium, Guignardia* and *Phomopsis* have high sequence similarities and are phylogenetically related to their saprotrophic counterparts. This provides evidence to suggest that some endophytic species change to a saprotrophic lifestyle. In addition, fungal diversity on pine needles was investigated based on morphological comparison coupled with a molecular approach based on DGGE and phylogenetics. Morphological and culture dependent studies showed that about 80% of fungi were anamorphic. PCR-DGGE analyses recovered 40 different fungal operational taxonomic units (OTU). Phylogenies indicate that 32 are Loculoascomycetes, 4 are phylogenetically related to mitosporic Letiomycetes, 2 are members of the Trichocomaceae and another 2 belong to the Hypocreales and Lecanorales respectively. A number of these fungi have not necessarily been recovered from morphological and cultural methodologies as well as from previous endophytic studies reported elsewhere. These taxa, which are morphologically and culturally undetectable undoubtedly play vital roles in living and partially decayed needles. These findings have important scientific implications in biodiversity and ecological studies.

S41PS1 - 0411

Endophytic fungi in non-mycorrhizal oak roots

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Endophytic fungi are well studied on above-ground portions of plants. However, only little is known on the endophytic colonization of plant roots and detailed investigations of healthy roots exist only for a few hosts. This study aimed to investigate the endophytic mycobiota in non-mycorrhizal roots of *Q. petraea* and *Q. robur*.

A detailed survey was conducted at two sites in eastern Austria. Sites were chosen to reflect differences in altitude, humus and soil type and pH within the natural range of oak in Austria. The investigated oak stands differed in age and species composition, with *Q. petraea* dominating at site 1 and *Q. robur* at site 2. At both sites roots were sampled from 3 healthy-looking and 6 declining oak trees. Excavation and sampling of roots was carried out from eight soil profile pits as well as from additionally collected soil cores. For fungal isolation, a subsample of 680 very fine (<0.2 cm), 340 fine (0.2-1 cm), 229 structural (1-3 cm), and 108 coarse roots (>3 cm) were taken, giving a total of 1357 root samples. After surface sterilization with ethanol (96%) and sodium hypochlorite (4% avail. chlorine), a 5-10 mm segment was cut from the middle of each 8 cm long root section, separated into cortex and central cylinder, and plated out on MEA (2%).

Overall fungal colonization of non-mycorrhizal oak roots was 97.7%. However, colonization frequency of the cortex was nearly twice that of the central cylinder. In total, the species assemblage comprised 119 fungal taxa. Species composition varied greatly between sites: At site 1, *Cystodendron* sp. (which likely represents a new species), a *Cadophora*–like species and *Crypto-sporiopsis radicicola* were the dominant species. Other frequently recorded species were *Mollisia* cf. *cinerea* and *Phialocephala fortinii*. At site 2, fungal community was dominated by *Cadophora fastigiata*, which was recovered from almost 50% of the root segments, with *Cylindrocarpon destructans* as the other major component. *Cryptosporiopsis melanogena* and *Phoma* cf. *radicina* were also frequently (>10%) isolated at this site.

The number of endophytic species isolated from roots was considerably higher compared to that obtained from above-ground lignified parts of oak. Variation of both species composition and frequency of species were much higher between sites than between oak species at the same site. Thus, between-site differences in edaphic and climatic conditions had a greater impact in determining the species community than the hosts. The endophytic microfungal community comprised primarily common soil fungi. However, saprobic rhizosphere fungi, fungal root pathogens, dark septate endophytes and fungi that form mycorrhizal associations were also obtained. Some of the frequently isolated species were found to form endophytic root associations with other perennial hosts.

S41PS2 - 0639

Metabolic and taxonomic approaches to investigating the effects of plant function on communities of root and nodule-associated fungi.

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The monitoring of biodiversity, including that of various fungal communities, is a growing priority. This study compares two methods that describe fungal communities, and thus may be used to evaluate their biodiversity. In addition, this work tests the hypothesis that the communities of endophytic fungi in equivalent tissues of functionally different plants in the same environment will be unique. This hypothesis has implications for studies using plant biodiversity as a proxy for endophytic fungal biodiversity.

Nodulated roots of *Alnus incana*, and a comparable, related, and cohabiting non nitrogen-fixing *Betula papyrifera* were collected from four ecologically similar sites in western Canada. Standardized homogenates prepared from surface-sterilized root tips and nodules from three samples per site were streaked on selective media to identify and enumerate culturable fungi. This homogenate was also pipetted into Biolog © microtitre plates to obtain substrate utilization profiles of the associated fungal communities.

Preliminary analyses show that while the substrate utilization profiles of all sample types overlap, there is more variability between those of root samples than those of nodule samples. Preliminary community composition data support these findings: fungal communities associated with nodules showed less interpopulation variability and were more distinct taxonomically compared to those associated with the roots of either plant species.

These preliminary observations show that the two approaches to describing fungal communities yield similar patterns, thus either approach may be sufficient for biodiversity inventories. These results suggest that plant functioning alone may not be sufficient to shift communities of root endophytic fungi, although nodules appear to have a localized influence.

S56IS1 - 0370

Phylogeography of Serpula lacrymans reveals global migration events and multiple transitions to an indoor lifestyle

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The dry rot fungus *Serpula lacrymans* (Basidiomycota) is the most feared destroyer of wooden buildings and constructions in temperate regions. It has a widespread distribution in buildings in temperate regions (Eurasia and North America), and causes also deteriorations in buildings in New Zealand and cooler regions of Australia. For a long time it has been an enigma from where the dry rot fungus first spread and colonized the human domain. In this study, a comprehensive sample of *S. lacrymans* has been assembled, including material from newly discovered localities in nature.
An extensive molecular dataset has been generated, including microsatellites, AFLPs and sequence data from three nuclear loci. In accordance with earlier studies, all our analyses demonstrate that *S. lacrymans* consists of two highly differentiated lineages; one mainly occurring in nature in Northern California (var. *shastensis*); and another group including isolates from all continents both occurring in nature and buildings (var. *lacrymans*). The analyses indicate that var. *shastensis* is genetically most similar to the ancestral lineage of the two forms. Var. *shastensis* has been found exclusively in high altitude mountainous regions and is possibly adapted to areas with high snow cover. In contrast to var. *lacrymans*, var. *shastensis* has never been detected indoors, and an important ecological transition has happened between the two lineages, enabling the switch to a largely indoor life style in var. *lacrymans*.
Our results demonstrate that var. *lacrymans* has an unrecognized widespread natural distribution in North East Asia. Var. *lacrymans* is genetically most variable in this region, indicating that North East Asia most likely represents the ultimate source population for var. *lacrymans*. The fungus has apparently colonized the human domain independently in Asia and Europe. The extreme genetic homogeneity in the indoor European population indicates that this population established through a recent founder event. Long distance dispersal events have happened to North and South America and Oceania, most likely from Europe. This study shows that a complex phylogeographic structure is observed in *S. lacrymans* caused by the interplay between natural migration and distribution patterns and more recent human mediated dispersal events.

S56IS2 - 0921

Biogeography of the Hysterangiales

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Hysterangiales is an order of ectomycorrhizal Basidiomycota that forms truffle-like fruiting bodies. It is distributed globally, both in the Northern and Southern Hemispheres, but each species is restricted to well-defined areas of endemism. Truffle-like fungi are mostly assumed to be incapable of long distance dispersal as their spores are only spread via small animal mycophagy. Based on both the high occurrence of endemism and limited spore dispersal, we hypothesized that the distribution of the order may be strongly influenced by vicariance. Multigene phylogenies resolved three major clades within the order that are composed exclusively of the Southern Hemisphere taxa, and they form a basal paraphyly, strongly supporting an origin of the Hysterangiales in the Southern Hemisphere. The results of ancestral area reconstructions are consistent with the hypothesis of an east Gondwanan, i.e. Australian, origin of the order. Although the topologies of some more terminal clades are consistent with vicariance (e.g., a sister relationship of New Zealand and New Caledonian taxa), some areas (e.g., Australia) are in several different subclades of the order, which is in conflict with a strict vicariant scenario. Therefore the importance of long distance dispersal, though probably a rare event, could not be discarded. Although a Cretaceous or younger origin of the order remains as a possibility, overall patterns indicate a Paleozoic origin of the Hysterangiales, which is much older than the oldest fossils of mushroom-forming fungi. This also indicates that the Hysterangiales could exist prior to the origin of the currently recognized ectomycorrhizal plants, as well as the arrival of mycophagous animals in Australia. This inconsistency between the estimated age of the Hysterangiales and the fossil record of its extant hosts suggest that either the three ectomycorrhizal clades of the Hysterangiales represent parallel evolution of the ectomycorrhizal symbiosis or that the Hysterangiales was mycorrhizal with members of the extinct flora of Gondwana. We will have thorough discussion of several biogeographical hypotheses on the Hysterangiales, including dispersal vs. vicariance, host-tracking vs. host-shifting, and the age estimate of the order.

S56IS3 - 0613

Hitchhiking through the botanic realm: Ustilaginales in time and space

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Smut fungi have been of great interest for a long time for they include economically important plant pathogens. The phylogeny of the group has been under discussion and the combination of ultrastructural and molecular data provided insight in the evolution of the heterogeneous group. The hirachic level of the group has been updated several times and the former Ustilaginales are now treated as Ustilaginomycotina and include several new orders. These studies have also revealed the enormous influence of host plants in the evolution of these parasites.
Our analyses of ultrastructural characters such as septal pores and interaction interfaces in combination with multiple gene molecular phylogenies resulted in new interpretations of the evolutionary trends in smut fungi. The exclusion of *Microbotryum* from the Ustilaginomycotina highlights a number of convergences of many important fungal traits in two subphyla. The crucial phylogenetic position of *Entorrhiza* is discussed based on additional characters, and our data implicate a new understanding of Basidiomycota. The combination of host and parasite phylogenies resulted in a better understanding of the evolution of Ustilaginales. The relevance of ecological niches and adaptations to host ecology could be demonstrated with our new data. The distribution of cospeciation and horizontal transfers are analysed to understand the coevolution of basidiomycetous plant parasites.

S56PS1 - 0304

A phylogenetic and phylogeographic approach to delimit Antarctic and bipolar species of the genus *Usnea*, Neuropogon

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Species of the lichen genus *Usnea* subgenus *Neuropogon* have their centre of distribution in polar regions of the Southern Hemisphere. Their morphological and chemical variability is poorly understood and several asexual taxa with uncertain relationships to fertile taxa occur in the group. The species concept of the group is uncertain. We combined a phylogenetic circumscription of *Neuropogon* species and the phylogeographic context for species delimitation and recent distribution of species. The importance of morphological and chemical variability within and between species was also investigated. Besides South American and Antarctic species also bipolar lineages exist. We examined potential causes of this distribution, in particular the question if there is genetic exchange between both polar regions and between South America and Antarctica. A dataset of more than 300 specimens and three gene fragments *(ITS, IGS and RPB1)* was used. The phylogenetic analyses revealed three related groups of mainly asexual lineages arranged around three fertile *Usnea* species. A phylogenetic species recognition method detected several cryptic species within these three groups and a bipolar lineage in two of the complexes, which were formerly described as a single species, *Usnea sphacelata*. The dataset of each group is used separately in a phylogeographic context performing nested clade analysis to infer species boundaries and population history on an intra-interspecific interface. The concordance of genetic deviation and geographical distance is overall small. All Northern Hemisphere populations are genetically very homogeneous compared to their Southern Hemisphere counterparts, which points to recent long-distance dispersal. There is broad evidence for cryptic speciation in South America and post-glacial recolonisation of Antarctica as well as periodic gene flow from South America into Antarctica. To support species delimitations the association of morphological and chemical characters with the position of haplotypes on hierarchically nested haplotype networks was reviewed using contingency tests and ANOVAs. In almost all cases phylogenetic and phylogeographical conclusions are significantly corroborated by these data.

S56PS2 - 0437

Migration in space and time for 14 worldwide populations of *Mycosphaerella graminicola*

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We wanted to test if using different marker systems would allow us to differentiate gene flow events over time,
DNA sequences from six nuclear loci and data from three microsatellites were collected from fourteen globally distributed populations of the plant pathogenic fungus *Mycosphaerella graminicola*. Haplotype networks were constructed for the six sequence loci and population subdivision was assessed using Hudson's permutation test. Several migration models all based the coalescence theory were used to estimate migration among populations within and among continents for both the DNA sequence loci and the microsatellite loci. While subdivision was detected among the six regional populations, directional gene flow indicated possible spread of the pathogen among regional populations. The European and Israeli populations contributed the majority of historical immigrants to the New World. Migration estimates for microsatellite loci were used to infer more recent migration events among specific New World populations.
We conclude that it is possible differentiate gene flow over time, and that gene flow plays an important factor in determining the demographic history of *M. graminicola*.

S43IS1 - 0822

Production and formulation of antagonists for improved competitiveness and biocontrol

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A prerequisite for the successful development/commercialisation of biocontrol agents (BCAs) is the production and formulation of products which have the necessary physiological quality, shelf-life and consistency of performance when use in terrestrial ecosystems. Thus a major hurdle to success has been the production of quality inocula with the necessary ecological competence. The objective of our approach has been to examined the use of physiological manipulation of the growth of fungal BCAs to channel or synthesise useful endogenous reserves which are implicated in improved environmental stress tolerance combined with conserved biocontrol capacity. Increased accumulation of trehalose has implications for desiccation tolerance, while compatible solute accumulation (glycerol, erythritol, arabitol or mannitol in fungi) can improve tolerance to water and temperature stress. Examples will be chosen from studies on biocontrol fungi including *Candida sake*, *Pichia anomala*, *Epicoccum nigrum* and *Ulocladium atrum*.
Studies were conducted with physiologically modified conditions to enhance synthesis of useful compounds. These were analysed by HPLC. The potential for conservation of these useful compounds was examined using isotonic solutions. Subsequent studies involved formulation of BCAs using fluidised bed drying to produce inocula. The performance of formulations were compared against fresh cells.
Studies showed that this approach significantly increased the quality and environmental tolerance of inocula. Isotonic solutions could conserve concentrations of endogenous reserves inside cells prior to formulation using fluidised bed drying. Modified conidia have been shown to have better virulence against insect pests; and formulations of yeasts have been shown to be as effective as fresh cells for controlling spoilage fungi and mycotoxins in moist cereals.
The potential for application of this approach will be discussed.

S43IS2 - 0842

Screening of biocontrol agents against fungal leaf diseases

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Fungal leaf diseases can potentially be controlled by antagonists preventing infections or reducing the formation of primary or secondary inoculum. Antagonism often is based on nutrient competition, antibiotic production or hyperparasitism. Candidate antagonists can be isolated from the habitat of the pathogen. For the choice of a control strategy and the selection of suitable antagonists, the whole life cycle of the pathogen should be considered. Potential antagonists may be found on the host plant interfering with the pathogen during its pathogenic stage, as well as on crop residues. Pathogen populations often depend on such crop residues for survival and multiplication in the absence of the host.
The antagonistic efficacy of candidates can be evaluated in bio-assays under controlled conditions and has to be confirmed under field conditions. For the development of a commercial biocontrol agent many selection criteria besides antagonistic efficacy have to be considered in such screening programmes. Important ecological criteria are the adaptation of antagonist candidates to the specific environmental characteristics of the phyllosphere such as rapid changes of water availability and UV-irradiation. Important economical criteria are amongst others the possibility of cheap production of antagonist inocula and a long shelf life of the antagonist in the formulated biocontrol agent. Considering legal registration of biocontrol agents, important criteria are possible toxin production by the antagonist candidates and other potential health risks for users and consumers.
Results from screening programmes aimed at the selection of antagonists of *Botrytis cinerea* (grey mould) or *Venturia inaequalis* (apple scab) will be discussed. The antagonist *Ulocladium atrum*, efficient against *B. cinerea* in crops such as grapevine, tomatoes and ornamentals under commercial growing conditions, shows many characteristics which explain the good performance in the phyllosphere, e.g. spore survival on leaf surfaces, rapid spore germination during short wetness periods in a broad temperature range and survival of germ tubes during repeated interruptions of leaf wetness periods. Antagonists of *V. inaequalis* reducing the formation of overwintering pseudothecia or suppressing conidiation on apple leaves during summer are currently screened in bio-assays or using DGGE as a molecular tool for fingerprinting microbial populations in apple leaves in relation to *V. inaequalis* development. All candidate antagonists have to pass a pre-screening and must produce a minimum number of spores (economical feasibility) and grow at 5° and at low water potential (ecological competence). Candidates growing at 36 °C or belonging to fungal genera with known potential to produce mycotoxins are excluded from the further screening.
For the selection of antagonists suppressing conidiation of *V. inaequalis*, 160 fungal isolated were obtained from apple leaves infected by the pathogen.
From these isolates, 80 passed the pre-screening. In bio-assays on apple seedlings, 12 isolates reduced the pathogen sporulation significantly. These 12 isolates are currently mass-produced and formulated. Those which will be suitable for commercial production will be tested under orchard conditions.

S43IS3 - 0987

Strategies to improve *Metarhizium* control of arthropod pests

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Several strains of the entomogenous fungus *Metarhizium anisopliae* are being developed for the control of arthropod pests (e.g. ticks, mites, weevils). They provide an environmentally friendly alternative to chemical pesticides which are being withdrawn or to which pests have developed resistance. However, *M. anisopliae*, like other fungal biological control agents, often acts slowly and gives inconsistent results in the field. Considerable progress has been made in recent years to improve the efficacy of *M. anisopliae*. For example, markers have been developed which can quickly tell if the pathogen has become attenuated (i.e. become unstable or declined in virulence). The markers offer cost-effective tools to monitor the quality of the fungus during mass production. Another area where progress has been made is the use of *M. anisopliae* with low dose chemicals. This approach gives immediate crop protection and at the same gives the pathogen more time to kill its host. The level of control is often the same as that of the pesticide used at the recommended rate.

S43PS1 - 0184

Trichoderma spp. and *Gliocladium catenulatum* associated with *Helicobasidium mompa* and *Rosellinia necatrix*

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Helicobasidium mompa and *Rosellinia necatrix* are soilborne plant pathogens. Their mycelia persist in the soil and spread from plant to plant on the root surface. Such a growth habit of both pathogens produces intense interactions with other microorganisms, including potential antagonists such as *Trichoderma.* On isolation of the pathogens from diseased roots, we collected isolates of *Trichoderma* as primary contaminants. *Gliocladium catenulatum* was occasionally present in *Trichoderma* cultures as a secondary contaminant. We examined the antagonism of both types of contaminants to *H. mompa* and *R. necatrix* to determine whether they were potential biocontrol agents or simply present concomitantly on the surface of the pathogens.

Of 50 isolates of primary contaminants each from *H. mompa* and *R. necatrix*, 36 and 34 isolates were identified as *Trichoderma*, respectively. The pathogens had different species composition. Species of *Trichoderma* from *H. mompa* consisted of *T. harzianum* (44.4%), *T. koningii* (16.7%), *T. hamataum* (13.9%), *T. atroviride* (8.3%), *T. viride* (2.8%), and unidentified species (13.9%). Species composition of *Trichoderma* from *R. necatrix* was simple, consisting of three predominant species, i.e., *T. atroviride* (38.2%), *T. harzianum* (29.4%), and *T. hamatum* (23.5%), besides unidentified species (8.9%).

The antagonism of the *Trichoderma* isolates was invariably evident on *Lupinus luteus* plants grown in a commercial, weathered lapillus potting medium (Kanuma soil) delaying disease development by two days as compared to control plots without *Trichoderma*. The antagonism of *Trichoderma* was, however, obscure when plants were grown in unsterile field soi. There was no obvious difference in the origin of *Trichoderma* isolates or between species. *G. catanulatum* did not affect the *Trichoderma-R. necatrix* interaction but reduced disease severity of carrots incubated in vermiculite to estimate virulence of *H. mompa* isolates. Dual cultures with *H. mompa* revealed the antagonism of *G. catenulatum*, inciting swelling of cell walls and granulation of cytoplasm in *H. mompa*. Mycelial mat of *H. mompa* became indented where conidial suspension of *G. catenulatum* was dribbled. Its antagonism was, however, suppressed by mixing 10 % of unsterile soil with vermiculite.

In conclusion, *Trichoderma* spp. and *G. catenulatum* are potential antagonists. However, under field conditions, they may simply present as contaminants of *H. mompa* and *R. necatrix* and are not useful as biocontrol agents. Since these pathogens produce persistent mycelia abundantly in soil, they are likely to have other specialized mycoparasites which antagonize *H. mompa* and *R. necatrix* under field conditions.

S43PS2 - 0718

Effect of antagonistic fruit-borne yeasts on pathogenic and saprophytic fungi

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Infection of grapes by *Botrytis cinerea* causes bunch rot which is usually a destructive process (sour or grey rot) resulting in loss of the whole crop. Under specific climatic conditions characteristic of a few wine region of the world, the *Botrytis*-infected berries undergo a beneficial process called noble rot. Wines made from the nobly rotten grapes are among the world's most famous sweet and dessert wines. During the development of noble rot, a broad spectrum of additional moulds, yeasts and bacteria also colonise the berries that may interact in the competition for substrates and nutrients. The purpose of this study was to identify yeasts among the secondary colonists that have antifungal activities.
Yeasts were isolated from nobly rotten ("botrytized") berries, subjected to taxonomic identification by molecular methods (e.g. PCR-RFLP of the ITS region of the rDNA, sequencing of the D1/D2 domain of the 26S rDNA, electrophoretic karyotyping, etc.) and tested for antifungal antagonism. The mechanism of the antifungal activity was studied on conidia and on growing hyphae. To test the potential of the antagonistic yeasts for post-harvest bioprotection, apples were cut and dipped first in the suspension of yeast cells and then in the suspension of *B. cinerea* or *Penicillium expansum* conidia.
The majority of yeasts isolated from the botrytized berries had no detectable effect on the test organisms (*B. cinerea* and various species of *Aspergillus, Penicillium, Mucor* and *Rhizomucor*). Antifungal activity was found in isolates belonging to *Metschnikowia, Aureobasidium* and *Cryptococcus*. The *Metschnikowia* isolates studied in more detail released a diffusible substance into the environment that inhibited the germination of conidia and the growth of the mycelium. Growth inhibition was usually associated with lysis of hyphae at the growing tips. With the exception of *Candida zemplinina*, all the non-antagonistic yeasts isolated from the grapes were resistant. The most active *Metschnikowia* strains drastically reduced but not inhibited completely the growth of moulds on apples.
Certain yeasts growing on/in nobly rotten grapes have strong antifungal activity and can be used for post-harvest bioprotection of fruits.

S43PS3 - 0154

Nematicidal Metabolites From Fungi

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Nematodes attack a wide variety of organism and parasitic nematodes are a major challenge to humans and agriculture. Recently, the side-effect on the environment or human health of many synthetic pesticides has led to a drastic reduction of efficient commercial nematicides which make the search of new natural nematicidals necessary and important. The pool of natural products is an important source for searching potent nematicides. Fungi have a profuse secondary metabolism and are a major source of biologically active natural products. In the past years, nematicidal compounds had been reported for many fungal products. Here, we investigate the kinds of nematode-toxic fungi, the structure and nematicidal activity of compounds isolated from nematode-toxic fungi. So far, about 240 species fungi of 126 genera which mainly belong to Ascomycota, Basidiomycota and Deuteromycota have been reported to possess nematididal activity by producing toxic compounds against nematode, and according to reports, about 165 compounds including 96 novel compounds with nematicidal activity have been isolated from 87 strains of nematode-toxic fungi. Their diversiform structures mainly belong to alkaloid, quinone, isoepoxydon, peptide, macrolide, terpenoid, fatty acid, diketopiperazine, aphthalene, simple aromatics and other kinds of compounds. These compounds have nematicidal activities towards different nematodes, and most of them have selective nematicidal activities. Up to now, no major commercial product based on the compounds isolated from fungi has been developed, but some exciting results have been obtained, e.g. new peptide omphalotin has been obtained from the fungus *Omphalotus olearius* and it's nematicidal activity is similar with the commercially available nematicide ivermectin, which indicates that it is possible to search the potent active compounds from fungi to exploit novel nematicides.

S44IS1 - 0026

Diversity of Xylanase and plant cell wall esterases in thermophilic and thermotolerant fungi

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This paper reports the functional diversity of xylanases and plant cell wall esterases in thermophilic and thermotolerant fungi. The multiple isoforms of xylanases and plant cell wall esterases were resolved by IEF and each of the eluted fractions was studied for the expression of diverse xylanase and esterases. In all 85 distinct xylanases and 84 esterases were resolved on the basis of their pI from 14 different strains of thermophilic and thermotolerant fungi (*Absidia corymbifera*, *Acrophialophora nainiana*, *Aspergillus caespitosus*, *Aspergillus terreus*, *Chaetomium thermophilum*, *Chrysosporium lucknowense*, *Emericella nidulans* var. *lata*, *Humicola fuscoatra*, *Humicola insolens*, *Malbranchea flava*, *Melanocarpus* sp., *Penicillium lagena*, *Thermoascus aurantiacus* and *Thermomyces lanuginosus)*. The xylanases were characterized for functionally diverse substrate specificity and catalytic activities against various substituted and unsubstituted xylan types (oat spelt xylan, OSX; larch wood xylan, LWX; rye arabinoxylan, RAX; wheat arabinoxylan, WAX) and debranched arabinan, DA). The xylanases active at alkaline pH were also identified and their pulp bleaching potential was studied. These fungi were also studied for the presence of diverse plant cell wall esterases. These esterases on the basis of their differential activity towards p-nitrophenyl esters (p-nitrophenyl acetate, p-nitrophenyl butyrate, p-nitrophenyl ferulate) were classified as acetyl esterases and esterases Type *I*, *II* and *III*. Few unusual esterases with high affinity towards p-nitrophenyl butyrate as compared to p-nitrophenyl acetate and active under alkaline conditions were also identified.

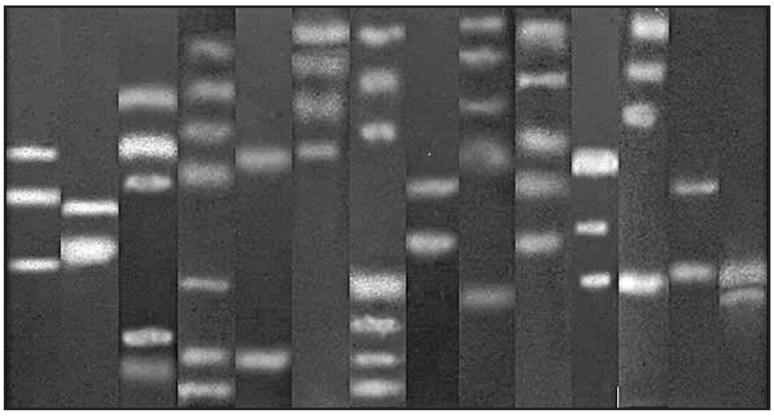

Fig. 1. Zymogram of PAGE gel representing xylanases produced by *Ab. corymbifera* (1), *Ac. nainiana* (2), *A. caespitosus* (3), *A. terreus* (4), *C. thermophilum* (5), *C. lucknowense* (6), *E. nidulans* var. *lata* (7), *H. insolens* (8), *H. fuscoatra* (9), *Melanocarpus* sp. (10), *M. flava* (11), *P. lagena* (12), *T. aurantiacus* (13) and *T. lanuginosus* strain D2W3 (14).

S44IS2 - 1007

New Fungal discoveries – of industrial relevance for biofuel and biopharma

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Use of Transposon assisted signal trapping (TAST) for screening of cDNA libraries has given many new hits of potential use in biomass conversion of e.g. left over agricultural products as wheat straw, corn, bagasse etc. Due to the TAST technology it is now possible to selectively go for the secreted proteins and also discover types of proteins for which we do not have high through put assays available. Progress and potentials will be illustrated by biofuel relevant examples.
The TAST technology has provided basis not only for enzyme discovery but also for peptide discovery in areas where only limited data are available. Of special interest is the recent discovery of a Cyanovirin-like compound. The first Cyanovirin was discovered several years ago and shown to have potentials as anti HIV/aids drug. However, Cyanovirin, being intracellular, was found to be difficult to produce large scale. We found a fungal variant of Cyanovirin and named Citrinovirin. The producing organism is *Penicillium citrinum* and it was here surprisingly found to be part of the secretome, having a signal peptide. Later also Cyanovirin-like compounds were found from other filamentous fungi (details will be presented).
Among fungal discoveries the Statin´s are one of the most significant discoveries since Penicillin, as Statin´s are the globally most significant type of drugs for cholesterol lowering in man. In collaboration between three research groups, Danish Technical University (Jens Frisvad and Thomas Ostenfeldt Larsen) and the Danish Hospital KAS Gentofte (Steen Stender), and Novozymes R&D a new type of Statin´s have been discovered. Data will be presented on its structure, derivatives and biological activity.
However, to enable commercial exploitation it is not sufficient to discover novel, interesting, and biologically active molecules. It is also important that they can be expressed in large scale in high yields. Several attempts have been made to express proteins from plants in filamentous fungi, which would allow the discovery pool for fungal large-scale produced products to be expanded significantly. Many such attempts of expression have surprisingly failed. A recent study has given new understanding of problems encountered with regard to heterologue expression of plant genes in filamentous fungi. Data will be presented.

S44IS3

Fungal cell wall biosynthesis and discovery of antifungals

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No abstract available.

1530-1730
SYMPOSIUM 45 - *Worldwide Movement of Fungal Forest Pathogens*

S45IS1 - 0569

Cryphonectria canker of *Eucalyptus:* A little-known disease caused by an assemblage of fungi of extreme quarantine relevance

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Cryphonectria canker causes one of the most serious diseases of *Eucalyptus* in plantations grown in the tropics and sub-tropics. Its impact has thus been responsible for shaping the nature of *Eucalyptus* forestry industry in many countries. DNA-based phylogenetic inferences have, in recent years, made it possible to review the taxonomy of the pathogen responsible for *Cryphonectria canker* of *Eucalyptus*. This substantially changed our understanding of the disease, its causal agent and its likely origin. *Cryphonectria canker* of *Eucalyptus* is now known to be caused not by a species of *Cryphonectria* but by a suite of species residing in the genus *Chrysoporthe*, which has been newly described to accommodate them. These fungi including *C. cubensis, C. austroafricana* and *C. doradensis* all have unique biological characteristics and they have clearly originated in different parts of the world. Interestingly, their hosts of origin are not species of *Eucalyptus*, but rather various tree species, not only the Myrtaceae, but more broadly in the Myrtales. *Chrysoporthe cubensis*, the best-known of the species is common in South East Asia and in South America and probably represents two different but closely related species. These fungi are all serious pathogens, some of which have already moved inter-continentally. They represent pathogens of great economic importance in countries where they are already known. Perhaps more importantly, in terms of quarantine, they appear to represent a highly threatening assemblage of fungi, which until very recently have virtually been overlooked.

S45IS2 - 0585

Global distribution and evolution of the pine pitch canker fungus, *Fusarium circinatum*

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Fusarium circinatum is an important pathogen of *Pinus* spp. that represents a significant threat to native and commercial forests, worldwide. This fungus causes large resinous cankers accompanied by pitch-soaked wood, crown die-back and stunted growth of established trees. The fungus can also be a very serious pathogen of nursery plants, where the symptoms of infection include root and root collar rot. Introduction of *F. circinatum* to new locations may be facilitated by insect vectors, although the majority of new introductions have probably resulted from trade in seed. Pitch canker is known in the USA, Mexico, Chile, Haiti, South Africa, Spain, and Japan, with unconfirmed reports from Italy, Iraq, South Korea and China. The primary objective of this study was to improve our understanding of the global distribution and spread of *F. circinatum* by studying its overall evolution and population biology. We, therefore, conducted a phylogenetic analysis that included representatives of *F. circinatum* populations from California, Florida, Mexico, Chile, Spain and South Africa. After DNA extraction, several housekeeping genes and non-coding regions were PCR-amplified and sequenced. These sequences were then aligned and subjected to phylogenetic analyses. Results revealed the presence of many nucleotide polymorphisms between the six populations, with fewer differences within populations. These data also allowed us to identify sequence signatures that differentiate some of the populations from others. Our preliminary data further suggest that isolates representing the Chilean isolates are more closely related to isolates from Mexico than to those representing the other populations examined. Ultimately the results of this study will provide valuable insight into the origin of the pitch canker fungus and its global spread.

S45IS3 - 0638

Microsatellite analysis documents worldwide and regional spread routes of the sudden oak death pathogen

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Sudden oak death is an emergent forest disease, caused by a recently described *Phytophthora* species. In this study, we use genetic analysis to elucidate the genetic structure of the pathogen in its known worldwide range, with the aim of understanding its spread routes and its reproductive biology. Analysis of twelve polymorphic simple sequence repeats identified in the genome sequence of *P. ramorum*, causal agent of 'sudden oak death', revealed three distinct clades among 151 isolates. Genotypic diversity was significantly higher in nurseries (91% of total) than in forests (18% of total). US forest and European nursery isolates clustered into two well supported and separate clades, while one isolate from a US nursery belonged to a third novel clade. Multilocus analysis determined populations in US forests reproduce clonally and are likely descendants of a single introduced individual. The genetic structure of populations from European nurseries displayed higher complexity, including multiple, closely related genotypes. All three clades were identified in some US nurseries, including genotypes that perfectly matched the US wild and the EU nursery genotypes, emphasizing the role of commercial plant trade in the movement of this pathogen. The combined microsatellite, sequencing and morphological analyses suggest the three clades represent distinct evolutionary lineages, all exotic to the US and probably Europe and of unknown origin. Analysis of over 200 additional isolates, using two hypervariable tetra-repeat microsatellite loci allowed for genotyping of asexually generated individuals. Results indicated different genotypes arise and dominate locally. In one case, evidence of replacement of old genotypes by newer ones was obtained. At times, genotyping allowed to substantiate possible spread routes for the pathogen. Our study documents the local adaptation of an introduced pathogen through the creation of novel genotypes via the accumulation of favorable mutations and/or somatic recombination

S45IS4 - 0270

Invasion of an exotic root pathogen of forest trees: the case of *Heterobasidion annosum*.

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Heterobasidion species are important root pathogens with circumboreal distribution. *H. annosum* was found to be associated with mortality of stone pine (*Pinus pinea*) in a Estate near Rome (Italy). This work reports on: i) how it was discovered that tree mortality was caused by a North American population of *Heterobasidion* probably introduced with infected wood during WWII, ii) the spreading of this exotic population, and iii) preliminary results on its interaction with an autochthonous *Heterobasidion* species.

Fruiting bodies collected in a mortality center in the Estate showed the presence of a mitochondrial insertion reported from North America, but known to be absent in Europe. This finding prompted us to sequence portions of the insertion and of three additional loci from the above fruiting bodies and 97 individuals of worldwide distribution. Maximum parsimony analysis was performed. Site history investigations were also conducted.

Using a systematic approach, *Heterobasidion airspora* was sampled in 15 forests along 280 km of coast approximately centered around Rome. Single-spore cultures were analyzed through PCR markers developed to differentiate European and North American mitochondria and nuclei.

Maximum parsimony analysis of the sequences showed that *Heterobasidion* fruiting bodies collected in the mortality center clustered within *H. annosum* individuals from eastern North America.

The exotic *Heterobasidion* species was found in all pinewoods within the 100 km range of expansion. In these forests, the exotic species was largely and significantly dominant, representing 98% to 100% of the total *Heterobasidion* inoculum. In a forest at the southernmost edge of expansion of the exotic population, both species were equally present. In this forest, the 2% of spores were nuclear-mitochondrial chimeras. The native *Heterobasidion* species was absent or present with low frequencies in the remaining forests located both within and outside the range of expansion of the exotic population.

Mortality centers are larger in the Estate than in surrounding forests, and this suggests the place where the introduction is likely to have occurred. The Estate has been closed to the public for centuries, but was occupied by US Army in 1944. The introduction could be linked to transport of infected woody military equipments. Data suggests a strong invasiveness of this exotic pathogen, displaying a potential rate of spread of 1,3 km/yr. Data also suggests that hybridization between the exotic and the native *Heterobasidion* species is occurring, but could be detectable only in forests where the two species are significantly present.

S45PS1 - 0320

Movement of the devastating *Eucalytpus* leaf and shoot pathogen *Phaeophleospora destructans*, throughout Asia

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Phaeophleospora destructans was first described in 1996 from north Sumatra, Indonesia, where it causes a severe leaf and shoot blight on *Eucalyptus grandis* in nurseries and young plantations. Since then it has been reported in nurseries and plantations in Vietnam and Thailand, with its host range extending to include *E. camaldulensis* and *E. urophylla. Phaeophleospora destructans* has also been reported from native *E. urophylla* in East Timor, presenting the possibility it may be native pathogen there. During surveys of nurseries in Southern China in 2004/2005, *P. destructans* was found to be widespread, including areas where there are no plantations, strongly suggesting that the pathogen is being transported throughout the region on infected germplasm. The ITS, beta-tubulin and elongation factor 1-alpha gene regions of three *P. destructans* isolates from each of Sumatra, Vietnam, Thailand and Southern China were sequenced and found to be identical. Microsatellite markers were developed for *P. destructans* using the FIASCO (fast isolation by AFLP of sequences containing repeats) enrichment technique and although 7 repeat-rich gene regions were identified, none of them were variable among the representative isolates tested. It appears that *P. destructans* in nurseries and plantations in Asia has very low genetic diversity. This could suggest a recent host jump from another species onto *Eucalyptus* or the introduction and movement of a very limited gene pool from Sumatra to the rest of Asia. *Phaeophleospora destructans* is currently absent from Australia but its devastating nature could potentially impact on biodiversity of native vegetation and productivity of *Eucalyptus* plantations and is thus considered a major biosecurity threat. The apparently low genetic diversity of the pathogen should reduce the risk of the impact that it could have on native ecosystems if introduced to Australia, butt plantations of susceptible trees would be at risk. A new initiative has recently been launched to test the susceptibility of native Australian eucalypt species to *P. destructans.*

S45PS2 - 0452

Phaeocryptopus gaeumannii and Swiss needle cast disease in New Zealand

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Phaeocryptopus gaeumannii, the causal agent of Swiss needle cast disease of Douglas-fir, has been present in New Zealand since the mid 1950s, where the disease has been responsible for substantial growth losses in plantations on both the North and South Islands. In northwestern North America, where the pathogen is native, the disease has been considered insignificant. However, since about 1990 an epidemic of increasing severity has been observed in Douglas-fir plantations near the Oregon coast. Based on measurements made since 1996, growth losses of 20 – 50% have been attributed to Swiss needle cast on about 150,000 ha of forest lands. Pathogen abundance and disease severity in western Oregon are correlated with mean daily winter temperatures and spring moisture. A model for prediction of disease severity based on these factors accounted for 77% and 78% of the variation in one- and two-year-old needles, respectively, for western Oregon sites. A similar disease prediction model is being developed to test the relationship between *P. gaeumannii* abundance and climate factors in New Zealand. The distribution of *P. gaeumannii* and severity of Swiss needle cast disease was surveyed in 16 Douglas-fir plantations throughout New Zealand in 2005. Retention of foliage was assessed in the field and samples of one- and two-year old needles were collected for assessment of *P. gaeumannii* abundance. These data will be used to derive a disease prediction model for Swiss needle cast in New Zealand that can be used to guide further research, perform climate change scenario analyses, and eventually to provide short and long term disease risk predictions and management cost/benefit analyses.

It has been presumed that spread of *P. gaeumannii* in New Zealand originated from a single introduction of the pathogen on the North Island in the early 1950s. This hypothesis is being tested using microsatellite markers developed for *P. gaeumannii* in North America. A preliminary comparison between *P. gaeumannii* populations from the North and South islands with North American populations indicates that the two New Zealand populations are related to widely separated populations in North America, and therefore may represent different introduction events. A more complete microsatellite analysis of the New Zealand *P. gaeumannii* population is being conducted using 1400 single spore isolates collected from 16 sites.

Friday 25th August 2006

Time	Activity				
07:30	**Registration** *Foyer*				
08:00	*Conference Room 1* **International Mycological Association (IMA) Board Meeting**	**Proffered Session 3** *Hall C* **Phylogeny 2** Clement Tsui (Australia)		**Proffered Session 4** *Hall D* **From Mycological Diversity to Phylogeny** Kálmán Vánky (Germany)	
10:00	**Coffee Break** – *Hall 2*				
10:30	**Symposium 46** *Hall C* **Anything Specific about Human Pathogens?** Alex Adrianopoulos (Australia) James Fraser (Australia)	**Symposium 47** *Halls A & B* **Biodiversity of Microfungi - A Phylogenetic Approach** Andrew Miller (USA) Amy Rossman (USA)	**Symposium 48** *Hall D* **Molecular Plant Mycorrhizal Interaction** Mark Tibbett (Australia) Paola Bonfante (Italy)	**Symposium 49** *MR 1 & 2* **Marine Fungi** Ka-Lai Pang (Hong Kong) Mohamed Abdel-Wahab (Egypt)	**Symposium 50** *MR 3 - 5* **Mycetozoan Biodiversity** Steven Stephenson (USA) David Orlovich (New Zealand)
12:30	**Lunch [pre purchase]** – *Hall 2*				
13:30	**Poster Session 5: Biodiversity and Conservation** **Poster Session 7: Industrial Mycology**				
14:30	**Symposium 51** *Hall D* **Finding the Missing Taxa: the Search for Fungi in Under-explored Habitats** Wendy Untereiner (Canada) James Scott (Canada)	**Symposium 52** *Hall C* **Fungi and Eucalypts** Eric McKenzie (New Zealand) David Ellis (Australia)	**Symposium 53** *MR 3 - 5* **Epidemiology of Fungal Pathogens** Wieland Meyer (Australia) Rosely Zancope-Olivera (Brazil)	**Symposium 54** *Halls A & B* **Fusarium - New Advances in Taxonomy, Biology and Detection** Brett Summerell (Australia) Keith Seifert (Canada)	**Symposium 55** *MR 1 & 2* **Conservation and Utilization of Fungal Biodiversity through Genetic Resource Centres** Pedro Crous (Netherlands) Akira Nakagiri (Japan)
16:30	**Coffee Break / "Clamp Connection" closes** – *Hall 2*				
17:00	**Plenary 5:** Mike Wingfield (South Africa) **Forest Fungi in a Changing World** *Halls A & B*				
18:00	**Closing Ceremony** *Halls A & B*				

0800-1000 Conference Room 1

International Mycological Association (IMA) Board Meeting

0800-1000 Hall C

Proffered Session 3: Phylogeny 2

Chair: Clement Tsui (Australia)

This session includes expertise having substantial knowledge in rusts,lichenized fungi, ectomycorrhizae, oomycetes, and etc., and provides a forum for phylogenetic studies that advance our understanding of fungal evolution. They will present latest classification scheme of different taxonomic groups inferred from phylogenetic trees constructed by various molecular sequence data. Also they will provide findings on or insights into evolutionary processes that led to current distribution of species.

0800-0820 PS1 - 0038

Lichen-forming pyrenomycetes are highly polyphyletic and not related to Sordariomycetes

H Thorsten Lumbsch (USA)

0820-0840 PS2 - 0305

Tackling phylogenetics in the large and diverse group of rusts of the family Pucciniaceae

Marlien M van der Merwe (Australia)

0840-0900 PS3 - 0622

Evolution of downy mildews

Markus Göker (Germany)

0900-0915 PS4 - 0504

The phylogenetic studies on the genus Cornumyces (Oomycetes) based on the nucleotide sequences of the nuclear large subunit ribosomal RNA and the mitochondrially- encoded cox2 genes

Shigeki Inaba (Japan)

0915-0930 PS5 - 0413

High level of gene flow and origin from native soil characterize Scandinavian populations of the soil borne fungus Penicillium scabrosum

Soren Banke (Switzerland)

0930-0945 PS6 - 0192

Phylogenetic classification and geographical patterns of species distribution in the ectomycorrhizal genus Cortinarius

Sigisfredo Garnica (Germany)

0945-1000 PS7 - 0390

A phylogenetic approach to accommodate Ramichloridium orphans

Mahdi Arzanlou (The Netherlands)

0800-1000 Hall D

Proffered Session 4: From Mycological Diversity to Phylogeny

Chair**:** Kálmán Vánky (Germany**)**

Within this session, selected aspects of mycodiversity and phylogeny will be presented. Such aspects are the diversity of the yeasts associated with flowers in Cuba, as well as the great number and diversity of smut fungi in Australia, of which the half is endemic. Phylogenetic aspects of the broad diversity of mycorrhizal associations involving members of the recently described heterobasidiomycetous order Sebacinales will be presented, as well as the molecular phylogeny of *Verticillium fungicola* and related taxa.

0800-0825 PS1 - 0382

Yeasts associated with flowers in Cuba

Heide Daniel (Belgium)

0825-0850 PS2 - 0624

NMR spectroscopy: a tool for rapid yeast characterisation and screening

Uwe Himmelreich (Germany)

0850-0915 PS3 - 0117

Australian smut fungi (Ustilaginomycetes), as surprising and diverse as the continent itself

Kálmán Vánky

0915-0940 PS4 - 0217

The expanding realm of the Sebacinales: basidiomycetes involved in a uniquely wide spectrum of mycorrhizal associations

Michael Weiß (Germany)

0940-1000 PS5 - 0093

Molecular phylogeny of Verticillium fungicola reveals its affinity with the genus Lecanicillium

Rasoul Zare (Iran)

1030-1230 **Hall C**

Symposium 46: Anything Specific about Human Pathogens?

Chair: Alex Andrianopoulos (Australia) / James Fraser (Australia)

An examination of the molecular mechanisms which govern growth and morphogenesis in human fungal pathogens and how these mechanisms impinge on pathogenicity.

1000-1030 IS1 – 0907

The Cryptococcus neoformans mating-type locus: Evolutionary insights from related species.

James Fraser (Australia)

1030-1100 IS2 – 1000

Expression profiles of Aspergillus fumigatus under human neutrophil attack and environmental stress

Gregory S. May (USA)

1100-1130 IS3 – 0998

Comparative genomic analysis of hypoxic stress response in Aspergillus fumigatus and A. nidulans

Kap-Hoon Han (South Korea)

1130-1200 PS1 – 0668

Identification of novel small molecule compounds that differentially inhibit the yeast form of Penicillium marneffei

Richard Kao (Hong Kong)

1200-1230 PS2 - 0784

Microarray analysis reveals genes responsible for the high virulence of the Cryptococcus gattii VGIIa Vancouver Island outbreak strain

Popchai Ngamskulrungroj (Australia)

1030-1230 **Halls A&B**

Symposium 47: Biodiversity of Microfungi - A Phylogenetic Approach

Chairs: Andrew N. Miller (USA) / Amy Y Rossman (USA)

With the tremendous advance in our understanding of the phylogenetic relationships of fungi, it is now possible to examine the biodiversity of microfungi by evaluating the completeness of taxon sampling within each taxonomic group. There also appears to be a number of putative new phylogenetic lineages being discovered which lie outside traditional groups. In this symposium, experts in major groups of ascomycetous microfungi will present the latest knowledge on the phylogenetics of their respective groups. Experts are encouraged to discuss the completeness of taxon sampling and the possibility of uncovering new phylogenetic lineages within each taxonomic group.

1000-1030 IS1 - 0775

Phylogeny and Biodiversity of the Hypocreales and Diaporthales

Amy Y. Rossman (USA)

1030-1100 IS2 - 0767

Phylogenetic relationships within the Helminthosphaeriaceae and Chaetosphaeriales

Sabine M. Huhndorf (USA)

1100-1130 IS3 - 0798

Phylogeny and Biodiversity of the Freshwater Euascomycetes

Carol A. Shearer (USA)

1130-1200 PS1 – 0649

Geomyces pannorum, a cosmopolitan soil fungus: phylogenetic relationships and species concepts

Sarah Hambleton (Canada)

1200-1230 PS2 - 0744

Unusual new species, exciting relationships – expecting the unexpected among woody decay pyrenomycetes from New Zealand

Toni Atkinson (New Zealand)

1030-1230 **Hall D**

Symposium 48: Molecular Plant Mycorrhizal Interaction

Chairs: Mark Tibbett (Australia) / Paola Bonfante (Italy)

Mycorrhizal symbiosis is an intimate association, usually mutualistic, between plants and fungi. Most terrestrial plants form one or more type of mycorrhizal symbiosis on or in their roots, and the fungi make up an important component of the ecology and biology of most soils. The symbionts engage in bilateral nutrient exchange where the plant receives mineral nutrients and the fungus obtains carbohydrates. Mycorrhizal research has entered the mainstream of biology, thanks mostly to DNA technologies and genomics, which are giving us new abilities to discover symbiont communication, development and diversity, and to reveal the contribution of symbiotic partners to the functioning of mycorrhizal associations. The aim of the symposium is to provide major insights derived from cellular, biochemical and molecular studies of mycorrhizal development, with focus primarily on arbuscular and ecto-mycorrhizas.

1000-1030 IS1 - 0754

Molecular signaling at early stages of the arbuscular mycorrhizal symbiosis

Natalia Requena (Germany)

1030-1100 IS2 - 0690

Transcriptional responses of Paxillus involutus and Betula pendula during formaton of ectomycorrhizal root tissue

Anders Tunlid (Sweden)

1100-1130 IS3 - 0706

Acquisition and long distance translocation of phosphorus in the symbiotic phase of arbuscular mycorrhizal fungi

Tatsu Ezawa (Japan)

1130-1200 PS1 - 0517

Pre-penetration apparatus: an arbuscular mycorrhiza-specific cell response in root epidermis

Paola Bonfante (Italy)

1200-1230 PS2 - 0023

Molecular identification of fungal endophytes in australian myco-heterotrophic orchids

John Dearnaley (Australia)

1030-1230 Meeting Room 1&2

Symposium 49: Marine Fungi

Chairs: Ka-Lai Pang (China)

Marine mycological research has spanned over a century. E.S. Barghoon and D.H. Linder are founders of marine mycology as their publication in 1944 has triggered significant interests in the field. Early research has focused on the morphological diversity of marine fungi on various substrata at different localities and since, more than 500 higher marine fungi have been described. Recent work has employed molecular techniques to tackle various taxonomic and ecological questions concerning phylogenetic relationships between taxa and molecular diversity on different substrata. On the applied aspects, marine fungi have been screened for bioactive compounds for medical uses and wood-modifying enzymes for bioremediation of organic pollutants. In this symposium, advancement in these areas over the years will be reviewed.

1000-1030 IS1 - 0121

Biodiversity of marine filamentous fungi and their phylogenetic relationships

Jariya Sakayaroj (Thailand)

1030-1100 IS2 - 0199

Documentation of marine fungal diversity: classical vs. molecular techniques

Ka-Lai Pang (China)

1100-1130 IS3 - 0058

Recognition of a caribbean marine fungus as a new genus by classical and molecular characters

Peter Mantle

1130-1200 PS1 – 0027

Metabolic profiles support species concept of two marine Dendryphiella species

Thomas E dela-Cruz (Germany)

1200-1230 PS2 - 0128

Morphological and molecular observations of Manglicola guatemalensis, a poorly known ascomycete

Satinee Suetrong (Thailand)

1030-1230 Meeting Room 3-5

Symposium 50: Mycetozoan Biodiversity

Chairs: Steven Stephenson (USA) / David Orlovich (New Zealand)

The mycetozoans (slime molds) consist of three phylogenetically distinct groups of eukaryotic, phagotrophic bacterivores once considered to be related to the true fungi and still almost invariably studied by mycologists. Although usually present and sometimes abundant in terrestrial ecosystems, only recently have efforts been made to assess global patterns of biodiversity for these organisms and the factors possibly responsible for these patterns. The papers presented in this symposium will report the results obtained from studies of the three groups of slime molds (myxomycetes, dictyostelids and protostelids) carried out throughout the world.

1000-1030 IS1 - 0155

Global diversity of cellular slime molds

John C. Landolt (USA)

1030-1100 IS2 - 0142

A global perspective on myxomycete biodiversity

Steven Stephenson (USA)

1100-1130 IS3 - 0294

Global distribution of the protostelids with particular emphasis on the deep Southern Hemisphere

Frederick W. Spiegel (USA)

1130-1145 PS1 - 0146

Dictyostelid cellular slime molds of Australia

Steven Stephenson (USA)

1145-1200 PS2 - 0144

Dictyostelid cellular slime molds from caves

John Landolt (USA)

1330-1430

Poster Session 5: Biodiversity and Conservation ***Exhibition Level***

Poster Session 7: Industrial Mycology ***Mezzanine Level***

1430-1630 **Hall D**

Symposium 51: Finding the Missing Taxa: The Search for Fungi in Under-explored Habitats

Chairs: Wendy Untereiner (Canada) / James Scott (Canada)

Estimates of the number of fungi vary widely, but it is now accepted generally that as many as 95% of fungal taxa are undiscovered and undescribed. While the highest proportions of new species are likely to be found in the tropics, it is evident that habitats supporting new fungi are worldwide in distribution. Unexplored habitats include marine and terrestrial plants, lichens, and insects but rocks, man-made structures, and vertebrates are also proving to be rich sources of new species. This symposium will examine research on a number of previously unstudied habitats harboring novel fungi as well as substrates yielding new species because of the application of techniques that favor the detection of rare or uncultured taxa.

1430-1500 IS1 - 0707

Fungi associated with marine wrack

David Malloch (Canada)

1500-1530 IS2 - 0799

Lessons learned from fungi associated with alcoholic beverage production

James Scott (Canada)

1530-1600 IS3 - 0719

Vertebrate-associated and keratin-degrading fungi from northern Canada

Wendy Untereiner (Canada)

1600-1615 PS1 - 0408

High throughput fungal culturing from plant litter by dilution-to-extinction

Gerald Bills (Spain)

1615-1630 PS2 - 0658

A novel widespread subphylum of Ascomycota unravelled from soil rDNA sampling

Terri McLenon (Canada)

1430-1630 **Hall C**

Symposium 52: Fungi and Eucalypts

Chairs: Eric H.C. McKenzie (New Zealand) / David Ellis (Australia)

All but a handfull of the 800 species of *Eucalyptus* are native to Australia, and over 200 of these have been introduced to other countries. The eucalypts (blue gums, stringy barks, ironbarks, etc.) dominate most landscapes within Australia. They form ectomycorrhizal associations with hundreds of fungal species; the foliage is attacked by many fungi; vapourised oils that create the blue haze rising above the Australian bush, also fuel fires that sweep through dry ecualypts; and some eucalypts are an environmental niche for the yeast-like fungus that causes cryptococcosis. These relationships between fungi and eucalypts will be covered within the symposium.

1430-1500 IS1 - 0676

How host specific are Mycosphaerella spp. infecting eucalypts?

Pedro Crous (The Netherlands)

1500-1530 IS2 - 0469

Mycorrhizal fungi and eucalypts - fungal significance in conservation and land management

Neale Bougher (Australia)

1530-1600 IS3 - 0708

Eucalypts as the natural host for the human pathogenic fungus Cryptococcus gattii

David Ellis (Australia)

1600-1615 PS1 - 0322

A reassessment of Phaeophleospora species on eucalypts

Vera Andjic (Australia)

1615-1630 PS2 - 0210

Fire and Fungi: survival, succession and composition of macro fungal community following fire in eucalypt forest in Western Australia

Richard Robinson (Australia)

1430-1630 Meeting Room 3-5

Symposium 53: Epidemiology of Fungal Pathogens

Chairs: Wieland Meyer (Australia) / Rosely Maria Zancopé-Olivera (Brazil)

In order to be able to response quickly to the emergence of pathogenic fungi and treat effectively fungal infections it is important to obtain knowledge of the epidemiology of pathogenic fungal agents. This symposium will review trends in the ecology, evolution, epidemiology and population genetics of selected important human (Paracoccidioides, Cryptococcus, Histoplasma, and Candida) and animal fungal pathogens (Batrachochytrium).

1430-1455 IS1 - 0879

***Paracoccidioides brasiliensis:* ecological and evolutionary aspects**

Eduardo Bagagli (Brazil)

1455-1520 IS2 - 1008

A global molecular epidemiological survey shows that the Vancouver Island outbreak strain is closely related to Latin American Cryptococcus gattii VGII isolates

Wieland Meyer (Australia)

1520-1545 IS3 - 0860

Molecular epidemiology of histoplasmosis: an update

Rosely Marai Zancopé-Oliveira

1545-1610 IS4 - 0760

National, population-based surveillance of candidemia in Australia with emphasis on disease acquired outside of hospitals

Sharon Chen (Australia)

1610-1635 PS1 - 0527

Enigmatic amphibian declines and emerging infectious disease: population genetics of the frog killing fungus Batrachochytrium dendrobatidis.

Jessica Morgan (Australia)

1430-1630 Halls A&B

Symposium 54:* Fusarium *- New Advances in Taxonomy, Biology and Detection

Chairs: Brett Summerell (Australia) / Keith Seifert (Canada)

Species of *Fusarium* cause some of the most economically significant plant diseases, and also produce several of the main mycotoxins subject to international regulation. Species concepts in this genus have always been controversial. This symposium presents several approaches to the delimitation and identification of *Fusarium* species using genetic, molecular and biochemical approaches, as well as examining the impact of several critical plant diseases.

1430-1500 IS1 - 0637

Genetic Diversity in* Fusarium *from Sorghum and Millet

John F. Leslie (USA)

1500-1530 IS2 - 0716

Development of an oligonucleotide array for detection of* Fusarium *species by hybridization of PCR products

André Lévesque (Canada)

1530-1600 IS3 - 0300

***Secondary metabolome – the bridge between phenetics and phylogenetics in* Fusarium**

Ulf Thrane (Denmark)

1600-1615 PS1 - 0427

Assays for rapid multiplex detection of toxigenic* Fusarium *spp. in cereals and derived products applying DNA array hybridisation and capillary SNP analysis, respectively

Arne Holst-Jensen (Norway)

1615-1630 PS2 - 0311

Origin and Diversity of* Fusarium *oxysporum f.sp. vasinfectum (Fov) in Australia

Bo Wang (Australia)

1430-1630 Meeting Room 1&2

Symposium 55: Conservation and Utilization of Fungal Biodiversity through Genetic Resource Centres

Chairs: Pedro Crous (The Netherlands) / Akira Nakagiri (Japan)

Fungal diversity, its extent and conservation, has in recent years been receiving considerable attention. Based on current estimates that suggest that there could be more than 1.5 million species of fungi, the question arises how genetic resource centres (GRCs) are approaching the issue of conservation of that portion of fungal biodiversity that is presently recognised. A further issue concerns how GRCs are providing ready access to specimens, cultures, DNA and associated data. This is important not only for immediate scientific needs, but also in view of future anticipated demands of fungal genomics and molecular biology, as well as conservation.

1430-1500 IS1 - 0773

The value of herbaria in the DNA age

Amy Y. Rossman (USA)

1500-1530 IS2 - 0788

MycoBank: linking names to genomes

Vincent Robert (The Netherlands)

1530-1600 IS3 - 0570

A global network of genetic resource centres to preserve fungal biodiversity

Joost A Stalpers (The Netherlands)

1600-1615 PS1 – 0549

Cooperation between biological resource centers (BRCs) in the CBD era – A challenge of NBRC and BRCs in Asia

Akira Nakagiri (Japan)

1615-1630 PS2 – 0525

Conservation and utilization of fungal biodiversity at BIOTEC Culture Collection (BCC) Thailand

Somsak Sivichai (Japan)

1700-1800 - 0750 **Halls A&B**

Plenary 5: Emerging fungal diseases threaten world forests

Mike Wingfield (South Africa)

1900 **Halls A&B**

Closing Ceremony

0800-1000
PROFFERED SESSION 3 - *Phylogeny 2*

PS3PS1 - 9938
Lichen-forming pyrenomycetes are highly polyphyletic and not related to Sordariomycetes
H.T. Lumbsch 1, I. Schmitt 1, R. del Prado1, S. Kautz 3, M. Grube 2
1 Dept. of Botany, The Field Museum, Chicago, IL, United States, 2 Insitut fuer Botanik, Karl Franzens Univ. Graz, Graz, Austria, 3 Fachbereich 9, Univ. Duisburg-Essen, Essen, NRW, Germany

The lichenized pyrenomycetes are outnumbered by discocarpous taxa, which also include the majority of macrolichens, while most pyrenocarpous lichens are crustose. Hence, most phylogenetic studies have concentrated on apotheciate lichen fungi, while pyrenocarpous taxa have largely been neglected. While traditional classifications of these fungi treated the lichenized forms separately with no or only superficial comparison to non-lichenized pyrenomycetes, classifications after the seminal work of Santesson (1952) aimed at integrating the lichen-forming pyrenomycetes into the system of ascomycetes. In these morphology-based classifications, lichenized pyrenomycetes were usually regarded as closely related to non-lichenized pyrenomycetes or loculoascomycetes. The majority of non-lichenized pyrenomycetes form a monophyletic group: *Sordariomycetes*. Interestingly, so far, none of the lichenized pyrenomycetes studied molecularly belongs to *Sordariomycetes,* which includes the bulk of non-lichenized pyrenomycetes. We studied the phylogeny of over 100 ascomycetes and the occurrence of lichenized pyrenomycetes in the fungal evolution, including most groups of lichenized pyrenomycetes, using gene sequences of three loci (nuLSU, mtSSU, RPB1). The lichenized pyrenomycetes are highly polyphyletic. Pyrenocarpous lichen-forming fungi occur in several lineages in each *Dothideomycetes*, *Chaetothyriomycetes*, and *Lecanoromycetes*, Most lichenized pyrenomycetes belong to *Chaetothyriomycetes* (such as *Pyrenulales*, *Strigulaceae, Verrucariales*), while only two families were shown to belong to *Dothideomycetes: Arthopyreniaceae* and *Trypetheliaceae*. Three families were shown to belong to *Lecanoromycetes*: *Porinaceae, Protothelenellaceae* and *Thelenellaceae*.

PS3PS2 - 0305
Tackling phylogenetics in the large and diverse group of rusts of the family Pucciniaceae
M.M van der Merwe 1, P.H Thrall 1, J.J Burdon 1, L Ericson 2, W Maier 4, J Walker 3
1 CSIRO Plant Industry, Canberra, ACT, Australia, 2 Department of Ecology and Environmental Science, University of Umeå,, Umeå, Sweden, 3 Forest Research and Development Branch, State Forests NSW, Beecroft NSW, Australia, 4 FABI, University of Pretoria, Pretoria, South Africa

The rust family Pucciniaceae is by far the most speciose group within the Uredinales, primarily due to high diversity in two genera: *Puccinia* (over 3000 described species) and *Uromyces* (over 600 species). These genera are separated solely on teliospore morphology. The hosts of these rusts are found in a wide range of Angiosperm families but prominent among these are the Poaceae, Cyperaceae and Asteraceae. Within both *Puccinia* and *Uromyces*, life histories include autoecious and heteroecious, macrocyclic and microcyclic rusts. While this group includes some of the most devastating crop pathogens, we still know little about the phylogenetic relationships and patterns among species in this group. Here we present phylogenetic analyses based on sequence data from three nuclear genes, beta-tubulin1-alpha, large subunit of ribosomal DNA and Translation Elongation Factor 1-alpha.
Sequences for a broad range of species within the Pucciniaceae were obtained and aligned using MAFFT. Alignments were used for the construction of phylogenetic hypothesis using Bayesian and Distance analysis. Host cladograms were constructed from the rust phylogenies and these were compared broadly with known phylogenetic relationships of the hosts as available from the Angiosperm Phylogeny group. The results reveal the presence of at least two distinct clades, possibly indicating two separate radiations that happened in parallel but within two different ecological/taxonomical host lineages. This supports the hypothesis that *Puccinia* radiated with the two large host families, Poaceae and Cyperaceae. Importantly, all of the analyses reject the hypotheses that *Puccinia* and *Uromyces* are monophyletic; species of both genera are found in various positions in both phylogenetic clades. Similarly *Endophyllum* is not a monophyletic genus. This indicates that classical taxonomical characters used for generic descriptions in the family Pucciniaceae are not useful for elucidating evolutionary relationships among species. While all genes used in this study provide valuable information the beta-tubulin gene was the most informative molecular marker and is furthermore a valuable marker at different taxonomical levels within the family Pucciniaceae.

PS3PS3 - 0622

Evolution of downy mildews

Markus Göker
University of Tübingen, Tübingen, Baden-Württemberg, Germany

Plant parasitism has independently evolved as a nutrition strategy in both true fungi and Oomycetes (stramenopiles). A large number of species within phytopathogenic Oomycetes, the so-called downy mildews (Peronosporales), could not be cultivated so far on any artificial medium and obviously are obligate biotrophic. Other genera like Phytophthora and Pythium can be more or less easily cultivated on standard or non-standard media. All three groups contain important plant pathogens which may cause severe economic losses. Albeit they seem to be an important model system to elucidate the evolution of obligate parasites, phylogenetic relationships between these organisms could not be sufficiently clarified so far.
The talk presents the recent insights into the phylogeny of downy mildews and their relatives based on molecular and morphological evidence. The focus will be on the relationships of obligate and facultative parasites, the interrelationships within downy mildews, and their ecological interpretation. Character analysis indicates an evolutionary scenario of gradually increasing adaptation to plant parasitism in Peronosporales and that at least the most important of these adaptive steps occurred only once, including major host shifts within downy mildews. The most important taxonomical changes will briefly be presented.

PS3PS4 - 0504

The Phylogenetic Studies on the Genus *Cornumyces* (Oomycetes) Based on the Nucleotide Sequences of the Nuclear Large Subunit Ribosomal RNA and the Mitochondrially- Encoded *Cox2* Genes

S. Inaba, S. Harayama
NITE Biological Resource Center (NBRC), National Institute of Technology, Kisarazu, Chiba Prefecure, Japan

The taxonomical position of the genus *Cornumyces* (Oomycetes) was inferred from analyses of the nuclear 28S rDNA and the mitochondrially- encoded *cox2* sequences.
The genus *Cornumyces* was erected by Dick (2001) for holocarpic oomycetous species infecting other oomycetes and plants, but its taxonomical position has still not been firmly established yet. *Cornumyces pygmaeus* (Zopf) M.W. Dick, former *Lagenidium pygmaeum* Zopf (Lagenidiales), is the only saprotrophic species of the genus associated with plant pollen.
In our floristic studies on Japanese Oomycetes, a species belonging to *Cornumyces* was identified in two soil samples obtained in Kanagawa Prefecture, Japan. The species was isolated from pine pollens used as baits in a mixture of the soil samples and sterilized distilled water. In pine pollens, it formed thalli that were ellipsoidal, non-septate, and non-branched. Sexual reproduction was also observed. The morphological characteristics were identical with those of *C. pygmaeus*. We tried to isolate them by using the direct plating method, and were able to obtain two axenic strains on peptone-yeast extract-glucose (PYG) and yeast extract-peptone-soluble starch (YpSs) agar plates. Sequence analysis of the 28S rDNA region showed that they clustered with leptomitaleous species such as *Leptomitus lacteus* and *Apodachlya brachynema*. On the other hand, a phylogenetic tree based on the *cox2* sequences indicated that they formed a cluster that was separated from all other oomycetous species examined.
In addition, we obtained an unknown oomycetous strain from a soil sample by using a piece of snake skin as bait. Nucleotide sequence of the 28S rDNA was almost identical with that of *Cornumyces* strains isolated from pine pollens. In the *cox2* region, however, 5.4 % differences were observed between the sequences of two *Cornumyces* strains isolated from different substrates. These results show that the strain from snake skin may be a new saprotrophic species of *Cornumyces*.

PS3PS5 - 0413

High level of gene flow and origin from native soil characterize Scandinavian populations of the soil borne fungus *Penicillium scabrosum*

S Banke 1, S Rosendahl 2
1 Biological Inst., Copenhagen, Denmark, 2 Biological Institute, Copenhagen, Copenhagen, Denmark

We investigated the population structure of the common soil borne fungus *P.scabrosum* and the dispersal among native and cultivated soils. Five DNA sequence loci were used to infer the population genetic history of 6 populations of *P. scabrosum* from Denmark and Sweden. Intergenic recombination among the 5 sequence loci were found using phylogenetic tests and clear indications of block structures was seen, when testing for compatibility among the variable sites. High levels of gene flow were found among all populations, indicating an efficient dispersal a asexual conidia up to 50 km.
Recombination was most frequent in the Danish populations isolated from native alkaline soil, the recombination events might be of older origin, as no known sexual structure is known for *P. scabrosum.* Fewer recombination events were observed in younger populations from cultivated soil, the findings of these recombination events could due to the high level of directional migration from the native soils.

PS3PS6 - 0192

Phylogenetic classification and geographical patterns of species distribution in the ectomycorrhizal genus *Cortinarius*

S. Garnica, M. Weiss, F. Oberwinkler
University of Tuebingen, Spezielle Botanik und Mykologie, Tuebingen, Germany

We inferred the phylogenetic relationships in the genus *Cortinarius* in a global scope integrating morphological and chemical traits of the basidiomes, as well as molecular phylogenetic analyses. Microscopical structures of the basidiomes were studied by light microscopy and basidiospore surface was examined by scanning electron microscopy. For a representative sampling of species of *Cortinarius*, internal transcribed spacers (ITS 1 and 2, including the 5.8S) and the D1/D2 (LSU) regions of the nuclear rDNA were sequenced and analyzed. To infer patterns of genetic and morphological infraspecific variation, we sequenced collections of the same species from different geographical origins. Additionally, we sequenced the domains A-C of the gene coding for the largest subunit of RNA polymerase II (RPB1) from selected species of each lineage. Several major clades appeared highly supported in the combined gene analysis. Major evolutionary trends and the character evolution of some specific *Cortinarius* lineages are discussed as well as aspects of global geographical patterns of species distribution.

PS3PS7 - 0390

A phylogenetic approach to accommodate *Ramichloridium* orphans

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The anamorph genus *Ramichloridium* presently accommodates a wide range of species with erect, dark, differentiated to slightly differentiated conidiophores and predominantly one-celled conidia. It comprises species with diverse life styles, including saprobes, human and plant pathogens. In the present study a *Mycosphaerella* species with a *Ramichloridium*-like anamorph was found associated with leaf spots on banana. In an attempt to describe this and other collections, a phylogenetic study was undertaken using all *Ramichloridium* species for which cultures are available at the Centraalbureau voor Schimmelcultures. The phylogeny derived from the ITS and 18S (SSU) rDNA sequences revealed *Ramichloridium* to be polyphyletic; five statistically supported clades could be distinguished. Several species (incl. *R. musae, R. pini*, *R. cerophilum, R. verrucosum* and *R. apiculatum*) formed a clade that resided in *Mycosphaerella*. The human-pathogenic *R. mackenziei* and *R. basitonum* grouped with other human-pathogenic taxa in the *Capronia* clade, together with the saprobic *R. anceps* and *R. fasciculatum. Ramichloridium obovoideum,* though morphologically similar to *R. mackenziei*, clustered with *Carpoligna* in the Sordariales. *Ramichloridium ellipticum, R. schulzeri* var. *flexuosum* and *R. schulzeri* var. *schulzeri* formed another distinct clade, related to the Pezizales. *R. epichloes* and *R. subulatum* represent the final clade, which was related to the Venturiaceae. Although previous morphological treatments distinguished two sections based on the nature of the conidial apparatus, it is clear from these findings that several un-described genera reside in what is currently seen as *Ramichloridium.*

PS4PS1 - 0382

Yeasts associated with flowers in Cuba

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Cuba is home to the largest remaining tracts of forest in the Caribbean and 15% of its area is under conservational management. The island harbours more than 6500 vascular plant species of which 50 percent are endemic [1]. Ecological studies from other regions than Cuba showed that the yeast communities associated with plants are determined by insect vectors and are specific and stable for a variety of habitats [2, 3]. To seek data on the global distribution of yeasts and to detect new species, a survey was undertaken to establish a baseline of flower-associated yeasts in Cuba.

Yeasts were collected from flowers of about 100 plant species in six of the 14 Cuban provinces between 2001-2004. From 168 samples, 195 yeast strains were isolated, representing 67 species including six potential new species and eight currently undescribed species that are known from other locations. Isolations from the same localities resulted more frequently in the same yeast species than isolations from the same plant species in different localities. With about 50% of the strains belonging to the Debaryomyces/ Lodderomyces clade and 20% to the Stephanoascus/ Metschnikowia clade [4], some species and phylogenetic groups were detected at higher frequencies than others, indicating their association with the investigated substrates. The detection of new and undescribed species was concentrated in the above two clades, demonstrating that the currently known species in these groups represent only a fraction of their diversity in the studied habitat.

Species accumulation curves were used to relate the number of detected species with the number of samples as a measure of the species density. They were calculated using a sample-based rarefaction function [5]. The curves for the four major collection areas and for all Cuban samples did not reach an asymptote. Extrapolated species accumulation curves revealed different levels of expected yeast diversity in the four sampling areas ranging from approx. 50 to 260 species. The species accumulation curves can be used as indicators in which areas the sampling should be continued to detect most of the existing species or, in general terms, to assess the completeness of a diversity survey. The resulting potential species density of an ecosystem is a crucial basis for conservational decisions.

[1] Center for applied biodiversity science: http://www.biodiversityhotspots.org /xp/Hotspots/caribbean/biodiversity.xml [2] Starmer WT, Schmedicke RA, Lachance MA (2003) FEMS Yeast Research 3: 441-448. [3] Lachance MA, Bowles JM, Starmer WT (2003) FEMS Yeast Research 4: 105-111. [4] Kurtzman CP & Robnett CJ (1998) Antonie van Leeuwenhoek 73: 331-371.[5] Colwell RK, Mao CX, Chang J (2004) Ecology 85: 2717-2727.

PS4PS2 - 0624

NMR spectroscopy: a tool for rapid yeast characterisation and screening

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Screening and identification of large numbers of yeast is essential for industrial, environmental and clinical applications. Increasing numbers of *taxa* and characters for their distinction are demanding on data management. Utilization of digitized data is of advantage for the characterization and identification of yeast.
NMR spectroscopy of whole cells has proven to be a rapid and robust technique to assess the phenotype of yeast [1]. These digital data allow the simultaneous determination of a large range of chemicals, the screening for particular metabolites and rapid assessment of the *metabolom.* The aim of this study was:
(1) to evaluate a hierarchical statistical classification strategy for a broad and extendable NMR-based identification and
(2) to evaluate NMR for screening of unknown, environmental yeast isolates.
A total of 1274 yeast isolates included the species Candida *albicans, C.dubliniensis, C.glabrata, C.parapsilosis, C.tropicalis, Clavispora lusitaniae, Cryptococcus neoformans, C.gattii, C.laurentius, C.humicolus, Issatchenkia orientalis, Pichia guillerimondii, Yarrowia lipolytica* and others.
NMR spectroscopy was performed on cell suspensions. A hierarchical identification system for clinical isolates was developed based on pair-wise classifiers [1, 2] that considered the taxonomic levels shown in Fig.1. This system was tested against pair-wise classifiers comparing all possible species combination seen in Fig.1 (requiring the development of an unfeasibly large number of 55 classifiers for the 11 taxa).

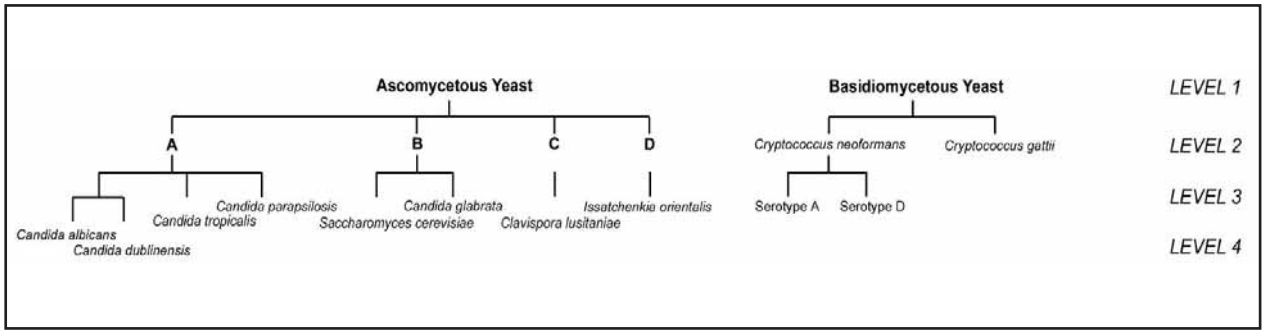

Fig.1: Hierarchical classification of the species utilized for the training set.
Similar accuracies were achieved when the conventional pair-wise classification (97% agreement with molecular identification) was compared with the hierarchical classification shown in Fig.1 (13 pair-wise classifiers, 95% agreement with molecular identification). The hierarchical system was also tested against species not included in the test set, but belonging to one of the "higher" taxonomic levels. In 90% of these cases, the respective isolates were assigned to the correct taxon, proving that this approach allows correct classification of species that were not part of the training process.
Further exploiting the potential of NMR, spectra of physiologically identical isolates of the genus Metschnikowia were compared by cluster analysis. Two well-separated clusters indicated the existence of distinct taxa, which were later confirmed by molecular tests, indicating the value of NMR spectroscopy for rapid screening in microbiology.
Statistical classification of NMR spectra is a suitable technique for rapid, robust and potentially automated characterisation and identification of yeasts that are otherwise difficult to distinguish.
[1] Himmelreich et al. Appl Environ Microbiol (2003) 69:4566-4574.
[2] Somorjai et al. in Artificial Intelligence Meth. & Tools for Syst. Biol. (2004) 5:67-85.

PS4PS3 - 0117

Australian Smut Fungi (Ustilaginomycetes), As Surprising And Diverse As The Continent Itself

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About half of the 290 known species of Australian smut fungi are endemic. The great ecological and morphological diversity of these endemic species is demonstrated by illustrating and considering some selected examples, including *Websdanea lyginiae* (on *Lyginia*), as well as some of the 20 known *Restiosporium* species that parasitise members of the 'southern rushes' (Restionaceae), which are represented in Australia by over 170 species. Two of the four known species of *Yelsemia, Y. arthropodii* (on *Arthropodium*, Liliaceae) and *Y. lowrieana* (on *Byblis,* Byblidaceae) are also endemic in Australia, as well as the unispecific *Fulvisporium* (on *Stipa,* Poaceae) and *Pseudotracya* (on *Ottelia,* Hydrocharitaceae). The type species of the genus *Heterotolyposporium, H. lepidospermae* (on *Lepidosperma,* Cyperaceae) is known only from Australia, as is the type species of the genus *Entylomaster, E. typhonii* (on *Typhonium*, Araceae). *Farysporium endortrichum* (on *Gahnia*, Cyperaceae) is known only from Australia and New Zealand. The diversity of the Australian smut fungi is further illustrated by consideration of endemic species in the genera *Entorrhiza* (*E. globigena, E. seminarii*), *Dermatosorus* (*D. schoenoplecti*), *Macalpinomyces* (*M. eriachnes*), *Moreaua* (*M. gymnoschoenii*), *Urocystis* (*U. chorizandrae*), *Sporisorium* (*S. paraneurachnis*), and *Ustilago* (*U. xerochloae, U. triodiae,* and *U. lituana*).

PS4PS5 - 0217

The expanding realm of the Sebacinales: basidiomycetes involved in a uniquely wide spectrum of mycorrhizal associations

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Within the basidiomycetes, the vast majority of known mycorrhizal species are homobasidiomycetes. It was therefore surprising that during the past years molecular and ultrastructural studies revealed a broad diversity of mycorrhizal associations involving members of the recently described heterobasidiomycetous order Sebacinales. It became evident that members of this order are involved in a wide spectrum of mycorrhizal types: ectomycorrhizas, orchid mycorrhizas (with autotrophic, mixotrophic or myco-heterotrophic orchids), mycorrhizas involving ericalean hosts, and also in associations with liverworts of the derived group of the Jungermanniales, which resemble mycorrhizas at the cellular level (jungermannioid 'mycorrhizas'). A comparably broad diversity of mycorrhizal associations is known from no other fungal group.

Sebacinales have recently also gained increasing interest because there is clear evidence from *in vitro* experiments with *Piriformospora indica*, an anomorphic member of the Sebacinales, that the interaction of fungi of this group with plant roots enhances growth, seed production and resistance against fungal pathogens in a phylogenetically wide range of host plants.

Sebacinales are phylogenetically divided in two distinct subgroups (informally designated as subgroups A and B), which correlates with the distribution of mycorrhizal types in which their members are involved. Ectomycorrhizal fungi (and, correspondingly, mycobionts of mixotrophic and myco-heterotrophic orchids) have only been detected in group A, whereas fungi involved in ericoid or cavendishioid mycorrhizas (a recently described mycorrhizal type found in certain epiphytic or hemiepiphytic Ericaceae) or in associations with liverworts as well as mycobionts of autotrophic orchids have only been found in group B. Basidiome-forming members are known only from group A. Teleomorphic individuals of group B have all been assigned to the *Sebacina vermifera* species complex.

We give an overview over the present knowledge concerning morphology and phylogenetic placement of the Sebacinales, present the results of new molecular phylogenetic analyses based on a comprehensive dataset of sebacinalean nuclear DNA sequences coding for the large ribosomal subunit (nrLSU), which includes many environmental sequences, and discuss conclusions from our molecular analysis related to ecology and evolution of the Sebacinales.

PS4PS6 -0093

Molecular phylogeny of *Verticillium fungicola* reveals its affinity with the genus *Lecanicillium*

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Verticillium section *Albo-erecta* was characterised by well-differentiated, erect conidiophores with verticillate arrangement of the phialides and mainly comprising fungicolous species. It was typified with *V. fungicola* (Gams & van Zaayen 1982). A large number of isolates around this species have been studied morphologically and molecularly (sequences of ITS regions and the small subunit ribosomal DNA). The isolates were also examined for their optimum growth temperature at eight different temperatures with three-degree intervals. According to ITS sequences, *V. fungicola* is close to the genus *Lecanicillium* W. Gams & Zare. *Lecanicillium* was characterized by having prostrate conidiophores including mainly entomogenous and also fungicolous species formerly classified under *Verticillium*. The value of temperature differences to separate the three varieties, *fungicola*, *flavidum* and *aleophilum*, is re-examined and compared with molecular findings which suggests raising the varieties to species level. The three varieties are indistinguishable based on their morphology, but they can sharply be separated based on maximum and optimum temperatures in addition to the ITS region. Other species formerly accommodated in sect. *Albo-erecta* are unrelated and require different generic classification.

1030-1230
SYMPOSIUM 46 - ***Anything specific about human pathogens***

S46ISI - 0907
The *Cryptococcus neoformans* mating-type locus: evolutionary insights from related species.
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Introduction: Sexual identity is governed by sex chromosomes in plants and animals, and mating-type loci (*MAT*) in fungi. In the basidiomycete yeast *Cryptococcus neoformans*, the *MAT* locus orchestrates production of basidiospores, the proposed infectious particle. Unlike most fungi where the *MAT* locus is <5 kb and contains 1 or 2 genes, in *C. neoformans MAT* is >100 kb in length and encodes over 25 genes.
Methods: We have isolated the *MAT* locus from several isolates of the most closely related species, *Cryptococcus gattii*, and other related species, including *Tsuchiyaea wingfieldii*, *Bullera dendrophila* and *Cryptococcus heveanensis*. Comparative analyses between these species have provided insight into the evolutionary events that fashioned this large, highly unusual region in *C. neoformans*.
Results: We hypothesise that the evolution of this structure began with sequential rounds of gene acquisition into two unlinked sex-determining regions, forming independent gene clusters involved in pheromone production/sensing and meiosis/karyogamy that later fused via chromosomal translocation. The *MAT* locus has since been subjected to intra- and interallelic gene conversion and inversions that suppress recombination, preventing the formation of hybrid mating-type alleles that would lead to sterility or self-fertility. These processes have resulted in multiple examples of pseudogenisation and gene loss, including the transition from each locus containing two homeodomain-containing sex-determining genes to only one.
Discussion: Together, these events resemble those that shaped mammalian and plant sex chromosomes, illustrating convergent evolution in chromosomal sex-determining structures in the animal, plant, and fungal kingdoms.

S46IS2 - 1000
Expression profiles of *aspergillus fumigatus* under human neutrophil attack and environmental stress
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The innate immune system has a central role in combating infections caused by *Aspergillus fumigatus*. Macrophages and neutrophils are two cell types of the innate immune system that are very important in preventing and eliminating *A. fumigatus* infections. Macrophages are the first line of defense, engulfing and killing inhaled conidia and possibly early germinating conidia before they can establish hyphal growth. Neutrophils attack and kill hyphae that invade tissue. The importance of neutrophils in combating and resolving infections caused by *A. fumigatus* is underscored by two observations. First, invasive aspergillosis disease is rare in people that have normal numbers of neutrophils, thus it is a disease primarily of a neutropenic host. Resolution of invasive aspergillosis disease strongly correlates with recovery of neutrophil numbers in patients that acquired disease while neutropenic. While these simple observations have been known for some time, we still know little about the interaction between *A. fumigatus*, and macrophages and neutrophils. We hypothesize that *A. fumigatus* responds to host cells of the innate immune system, macrophages and neutrophils, with specific transcriptional responses to defend against attack by these immune cell types, ultimately contributing to fungal virulence.
To begin to study the relationship of the interaction between neutrophils and *A. fumigatus* and virulence, we have undertaken a study of the global patterns of gene expression in *A. fumigatus* hyphae in the presence of human neutrophils in culture. We have identified the mRNAs with the most or least relative abundance when compared to hyphae in cell culture medium lacking neutrophils over a 60 minute time course. There are 532 *A. fumigatus* genes including 20 transcriptional regulators whose mRNAs are more abundant or less abundant by a factor of two fold or more, 428 more and 104 less. We have explored the expression pattern of these *A. fumigatus* genes under conditions variably related to virulence including H2O2 oxidative stress, osmotic stress, anaerobic stress, and glucose to sorbitol carbon source shift. Our analysis of the pattern of expression of these genes reveals information on the conditions imposed by the neutrophils on *A. fumigatus* hyphae and on the survival strategy of the fungus in responding to these killing conditions. Interestingly, some of the same mRNAs that are increased in response to the presence of human neutrophils are increased in response to one or more of the stress conditions. We have also examined these changes in mRNA levels in *sakA* and *mpkC*, MAP kinase gene deletion mutants, and determined that some of these changes are MAP kinase dependent. Overall these studies are providing a comprehensive view of the MAP kinase dependent changes in mRNA levels in response to specific stress conditions and human neutrophils.

S46IS3 - 0998

Comparative genomic analysis of hypoxic stress response in *Aspergillus fumigatus* and *Aspergillus nidulans*

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Aspergillus fumigatus is known as the primary causative agent of aspergillosis, which is an opportunistic fungal disease mainly localized in the respiratory system of human. Although the patients suffered from the invasive aspergilosis as well as the allergic diseases are getting increased, molecular mechanism of *A. fumigatus* infection has not been well elucidated yet. Currently, *A. fumigatus* has no known sexual development process, while *Aspergillus nidulans*, which is a very close relative of *A. fumigatus*, undergo complete sexual development process. Furthermore, in *A. nidulans*, hypoxic condition is the one of most important environmental factor of which the fungus preferentially generate fruiting bodies. The hypoxic condition is also important to *A. fumigatus* because of the environment of host cell is usually maintained as hypoxic condition. To study relationship between hypoxic stress condition and fungal virulence, comparative DNA microarray experiment was performed using *A. fumigatus* and *A. nidulans* microarray chips which were provided by pathogenic fungi genome resource center (PFGRC). As a result, we identified 4,332 reliable genes and, among them, isolated 362 hypoxic condition specific up- or down-regulated genes in *A. fumigatus* genome. Northern analysis was performed for validating the microarray analysis. Comparison between *A. nidulans* gene set provided information of shared and distinct pathway of hypoxic stress response and sexual development process. Duplicated analysis using *A. fumigatus* microarray ver. 2 chipset and relation of hypoxic stress and sexual development pathway will also be discussed. This work was supported by grant from KOSEF (R1-2006-000-11204-0) and KRF (KRF-2005-070-C00123).

S46PS1 - 0668

Identification of novel small molecule compounds that differentially inhibit the yeast form of *Penicillium marneffei*

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The emergence of *Penicillium marneffei* as a significant fungal pathogen particularly among human immunodeficiency virus (HIV) infected individuals living in Southeast Asia and southern part of China (including Hong Kong) poses a clear and immediate threat to these already heavily burdened regional public health systems. *P. marneffei* is the only thermally dimorphic species in its genus. It has been well speculated that thermal dimorphism of fungal pathogens is closely linked to their virulence. Our recent acquisition of a validated high quality diverse chemical library (50,240 small molecules with drug-like properties) and automated robotic platforms for high-throughput screening (HTS) enabled us to tackle virulence and dimorphism in *P. marneffei* using chemical genetics- the use of high-throughput screening (HTS) technologies to identify biologically active small molecules that will interfere with particular cellular processes/biological pathways/gene products in an organism.
Construction of an EGFP-expressing *P. marneffei* strain: Yeast cells of *P. marneffei* were grown at 37 oC to 2x108 CFU/ml in RPMI 1640 media. Cells were harvested and transformed with EGFP gene. *P. marneffei* transformants with plasmid gGFP integrated stably into the genome were selected on hygromycin B containing media.
Small-molecule compounds interfering with the growth of *P. marneffei in vitro*: EGFP-expressing strain of *P. marneffei* (104 cells/well) were cultured in RPMI 1640 at 37 oC in 5% CO2 in 384 well microtitre plates. 50,240 small-molecule compounds from the chemical library were transferred to each assay well using fully automated robotic platforms. The growth of the *P. marneffei* was monitored by GFP fluorescence
We have identified small molecule compounds that would specifically inhibit the 37 °C growing yeast form of *P. marneffei* but not the 25 °C growing mould form. The whole set of screening data will be presented and the compounds that selectively inhibited the yeast form at low micromolar concentrations without apparent inhibitory activity against mould form will be highlighted.
We are exploring novel strategies that can be employed to dissect complicated biological pathways involved in fungal pathogenesis. Compounds that interfere with specific growth phases of the fungus were successfully identified. The establishment of the first model of forward chemical genetics in *P. marneffei* will open new possibilities for the investigation of the pathogenesis of other model pathogenic fungi such as *Aspergillus fumigatus* and *Cadida albican*.

S46PS2 - 0784

Microarray analysis reveals genes responsible for the high virulence of the *Cryptococcus gattii* VGIIa Vancouver Island outbreak strain

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C. gattii, previously known as *Cryptococcus neoformans* var. *gattii*, is a pathogenic basidiomycetous yeast, which causes meningo-encephalitis in immunocompetent hosts. A specific major molecular type of this species, VGII, is responsible for an ongoing outbreak of cryptococcosis amongst humans and a range of animal species on Vancouver Island, Western Canada. Molecular typing has identified two subtypes, VGIIa and VGIIb, causing the outbreak with VGIIa being the vast majority of all isolates. All isolates are MATalpha. PCR-fingerprinting, AFLP and MLST studies have shown that both subtypes relate to isolates from a number of places around the world. Previous studies have shown that the VGIIa isolate, R265, is more virulent than VGIIb isolate, R272, in murine model and appears to be a parental strain for R265. In order to identify genes responsible for the greater virulence composite in R265 compared to R272, we used microarray analysis to compare the transcriptome of R265 and R272.
R265 and R272 were grown in minimal broth at 37ºC for 1 hour. RNA was extracted by Trizol reagent (Invitrogen) and purified by Qiagen RNeasy mini kit. RNA from both strains, grown in 8 different conditions (Growth in YPD broth at 30ºC, 37 ºC and 39 ºC; in 0.1%glucose YNB at 37 ºC; in 0.1%glucose YNB without amino acid and ammonium sulfate at 37 ºC; in RPMI with 54mM MOPS pH7.3 at 37 ºC; in RPMI with 29mM NaHCO3 and 25mM MOPS pH 7.3 at 37 ºC and in RPMI with 54mM MOPS and 0.75M NaCl pH7.3 at 37 ºC) was pooled together and used as the reference pool. Reference and experimental RNA from each strain, direct-labeled with Cy3 and Cy5 respectively, were hybridized onto a 70 mer oligonucleotide DNA microarray of strain JEC21 (serotype D) and genes of the mating types loci, a and alpha, from all serotypes. The experiments were independently performed in triplicates. Data acquisition was performed using the Axon GenePix Pro 4000A interface. Data were analyzed with GeneSpring GX version 7.3.1 using a p value < 0.05.
108 genes were up-regulated in R265 compare to R272. Several genes involved in putative virulence factors (e.g. *LAC1, LAC2, CAP64, MPK1*) and those involved in cell wall synthesis and iron absorption were induced in the virulent strain, R265. In contrast, 94 genes were suppressed in R265, these included genes involving in mitosis and ergosterol biosynthesis.
The results show that the transcriptome is different between two wild-type strains with dramatic differences in virulence. Genes responsible for major virulence factors such as capsule production, melanin synthesis and growth at high temperature, were up-regulated in R265 vs. R272. These findings correlate with previous studies revealing the higher virulence of the VGIIa isolate compared to VGIIb isolate. On the other hand, the reason for down-regulation of genes involving in cell division without any evidence of in vitro growth rate differences between strains is unclear. With this set of regulated genes, it will now be necessary to determine if there are differences in gene expression impact of selected genes on the virulence composite.

1030-1230

SYMPOSIUM 47 - *Biodiversity of Microfungi - A Phylogenetic Approach*

S47IS1 - 0775

Phylogeny and biodiversity of the *Hypocreales* and *Diaporthales*

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Over the past decade much progress has been made in understanding the phylogeny of the two major pyrenomycete orders, the *Hypocreales* and *Diaporthales*. Families have been accurately outlined and genera defined within each order. In both orders some genera and species exist that do not fall into any known family. Are these monotypic lineages or simply representative of unsampled groups? With increased sampling species or genera that were initially considered aberrant have clustered with related species and genera that are now regarded as families. However, discovery of synapomorphies has been difficult as obvious morphological characters are shown not to be phylogenetically informative. Multigene phylogenies combining nuclear ribosomal genes with protein genes are especially useful in resolving these relationships. Both the *Hypocreales* and *Diaporthales* are rich in asexual states with some lineages composed almost entirely of asexually reproducing species. Within genera the number of species has increased greatly especially when the asexual states are included as in such well-studied groups as *Bionectria-Clonostachys, Calonectria-Cylindrocladium*, *Hypocrea-Trichoderma* and *Gnomonia-Discula*. In most cases the sexual state is conserved morphologically with the greatest variability expressed in the asexual state. Conscientious collecting has revealed connections between sexual and asexual states suggesting that many supposed exclusively asexual species have rarely produced or cryptic sexual states. Field work combined with sequence data has revealed many previously undescribed species.. In general the more a group is studied the greater the number of species discovered. For most genera among these little sampled microfungi the asymptote of the discovery curve has not yet been reached. In addition sequence data of species complexes reveal the existence of previously unrecognized, morphologically cryptic species. These new species, once freed from the yoke of morphological constraint, sometimes turn out to have useful biological properties not manifested by the oppressor name. These well-defined species of *Trichoderma* have been shown to be useful such as in the biological control of cacao pathogens. New species and new lineages within phylogenetically complex species are being discovered as endophytes in the trunks of woody tropical crops. This research demonstrates that accurately defining genera and species in such economically important orders of fungi such as the *Diaporthales* and *Hypocreales* is the first step in controlling the diseases they cause or using them in biological control.

S47IS2 - 0767

Phylogenetic relationships within the Helminthosphaeriaceae and Chaetosphaeriales

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A number of taxa currently or previously placed in the Sordariales possess morphological characters similar to those found in members of the Helminthosphaeriaceae and Chaetosphaeriales. In an effort to better understand relationships among these taxa, a dataset of one or more genes was analyzed to determine their phylogenetic affinities. Portions of the nuclear 28S large-subunit or beta-tubulin gene, or both, were sequenced for numerous Sordarialean taxa, as well as several unnamed taxa. Subsequent phylogenetic analyses indicate many of these taxa form a well supported clade that can be recognized as the family Helminthosphaeriaceae. The ascomatal vestiture was found to be an important morphological character uniting taxa within the group. Several other taxa were placed outside this clade, but within an overall monophyletic group that contained the Helminthosphaeriaceae which is recognized as the Chaetosphaeriales sensu lato. These taxa possess a disparate array of morphological characters. The Chaetosphaeriales sensu lato may represent a series of evolutionary events where there was a large, rapid radiation of taxa with numerous morphological characters that presently provide only cryptic information about relationships.

S47IS3 - 0798

Phylogeny and Biodiversity of the Freshwater Euascomycetes

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Freshwater Euascomycetes have been studied intensively only over the past 30 years and current studies continue to yield many more taxa. Numerous new species, genera and lineages have been discovered. Currently, 530 meiosporic species of Euascomycetes have been reported from fresh water. These species are distributed among 20 of approximately 55 extant orders of
Euascomycetes. Among these 20 orders, freshwater species are, for the most part, concentrated in relatively few orders: Helotiales (101 spp.), Pleosporales (94 spp.), Sordariales (82 spp.), Melanommatales (33 spp.), Halosphaeriales (29 spp.), Eurotiales (25 spp.) and Jahnulales (18 spp.). Thus it appears that only certain evolutionary lines within the Euascomycetes flourished in freshwater habitats. We predict that more species in these lineages will be discovered over time. A large number of taxa, approximately 70, are considered *incertae sedis* and cannot be accommodated in any existing orders or families. There are also a number of singletons (collected only once) that differ in morphology from known taxa of Euascomycetes. Whether or not these singletons are rare species of known lineages or represent new lineages has not yet been determined. Several interesting examples of singletons that we now know to be new lineages already exist. *Jahnula aquatica* was first described in 1936. This genus remained monotypic until 1999 when five new species were described from submerged wood. Currently, *Jahnula* represents a new lineage based on molecular and morphological data, and the clade contains three genera and 13 species. *Ceriospora caudae-suis*, a species described from a lake in 1951, was placed in the genus *Ceriospora* with reservation. Little was known of this species until collections from North America revealed it to be one of the most common freshwater species in the USA. Phylogenetic analyses of molecular sequence data indicated that this species is a member of a newly discovered lineage of freshwater euascomycetes, the Annulatascaceae. Few teleomorph/anamorph connections have been made for freshwater meiosporic and mitosporic euascomycetes. Use of phylogenetic analyses of molecular sequence data, however, has facilitated reconciliation of freshwater mitosporic and meiosporic taxa. Estimated phylogenetic relationships among freshwater, terrestrial and marine euascomycetes, including both mitosporic and meiosporic taxa, will be presented for major lineages of freshwater euascomycetes

S47PS1 - 0649

Geomyces pannorum, a cosmopolitan soil fungus: phylogenetic relationships and species concepts

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The anamorph genus *Geomyces* Traaen is commonly isolated from soil and air samples, but has been reported also from feathers, from biofilms (degrading soil-buried polyester polyurethane), and from cutaneous material (though whether it causes superficial skin or nail infections is in dispute). The four species originally described by Traaen, differing primarily in colony colour, were placed in synonymy with *Chrysosporium pannorum* (= *Sporotrichum pannorum* Link). The genus was later recognized as distinct based on the formation of chains of arthroconidia on erect conidiophores rather than on development of aleurioconidia on short pedicels or directly on the sides of hyphae as occurs in *Chrysosporium*. Because Traaen did not designate a type species, *G. auratus* was chosen as lectotype species. Three additional species have since been described in the genus, but some authors have considered two of these as varieties of *G. pannorum*.

To assess conspecificity of strains of *G. pannorum* deposited in the Canadian Collection of Fungal Cultures (CCFC), and infer their phylogenetic affinities, partial nuclear ribosomal DNA sequences were determined and subjected to parsimony analyses. For morphological comparisons, strains were grown on oat agar (OA). Data for the type strains of *G. auratus*, *G. asperulatus*, and *G. pulvereus*, as well as strains of the teleomorphic *Pseudogymnoascus roseus* (Myxotrichaceae) (anamorph *G. vinaceus*), were included.

All isolates, except for *G. pulvereus*, were resolved in three strongly supported clades. Genotype groupings correlated with morphological colony types on OA. A majority of strains grouped in one large clade, comprising several sub-clades including one corresponding to *P. roseus*. Clade 2 comprised a single culture from wheat field soil and several sequences from biofilms. Clade 3 included the type strains of *G. asperulatus* and *G. auratus* and some strains identified as *G. pannorum*.

Considerable plasticity in colonial phenotype has been noted among isolates of the *G. pannorum* complex and there has been debate about whether observed variation merits species distinction. Our results suggest that Traaen's recognition of four species based on colour may be valid. Detailed studies of conidiogenesis may uncover additional morphological characters that can be used to recognize the phylogenetic taxa revealed by ITS sequence analysis. Small subunit rDNA sequences confirm previous results that *G. pannorum* and *P. roseus* are allied to the Leotiomycetes, while *G. pulvereus* was found to be related to the Onygenales.

S47PS1 - 0744

Unusual new species, exciting relationships – expecting the unexpected among woody decay pyrenomycetes from New Zealand

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New Zealand's woody decay pyrenomycete flora has been little studied. The cosmopolitan Lasiosphaeriaceae, largest and least studied family within the Sordariales, has long been noted for its morphological diversity and the artificiality of its grouping. This first systematic study of the Lasiosphaeriaceae in New Zealand uses morphology and phylogenetics to elucidate relationships within the New Zealand flora and facilitate comparisons with temperate relatives worldwide. In the course of this, attractive new species found within the Chaetosphaeriaceae highlight convergent evolution in these previously unified families. Traditional morphological characters such as ascospore shape and peridial wall structure provide convoluted and frequently conflicting evidence in the light of phylogenetics. The new species and species complexes illustrated suggest a high level of microfungal endemism exists on these relatively isolated islands.

S48IS1 - 0754

Molecular signaling at early stages of the arbuscular mycorrhizal symbiosis

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Plant roots release molecules that activate arbuscular mycorrhizal (AM) fungi and initiate the recognition process in the symbiotic fungus. In the absence of those signals, AM fungi are not longer able to grow asymbiotically and retract their protoplast back to the mother spore. GIN1 a novel AM fungal two-domain protein exclusively expressed during this early developmental stage, is shown here to be post-translationally modified by mycorrhizal lipids. The protein, with a self-splicing domain at the C-terminus, homologue to Hint domain of animal hedgehog proteins, is able to undergo self-splicing. Hint domain of metazoan proteins is responsible for the autoproteolytic activity of these proteins, releasing the mature N-terminal domain after attaching a cholesterol moiety at its carboxy end. This cholesterol modification determines the proper localization of the mature protein. We hypothesized that the Hint domain of GIN1 would also be able to induce self-splicing and to release the N-terminus modified with a sterol adduct. Indeed, in vitro analyses showed that Gin1-C is able to undergo splicing after addition of small nucleophiles such as DTT as also shown for hedgehog proteins. To determine which molecule would provoke the splicing in vivo, splicing assays with cholesterol or lipid extracts from spores, external mycelium and mycorrhizal roots were performed. Interestingly, only sterol extracts from mycorrhizal roots were able to induce splicing. We have nail it down to find out that several plant isoflavonoids, are able to induce the splicing of the fungal protein. The N-terminus of GIN1 shares similarity with the GTP binding protein family (IAN) evolutionary conserved from plants to humans. They are related to the control of local host defense against pathogens. In vitro experiments with recombinant GIN1-N showed that the protein has ATPase activity rather than GTPase. Localization experiments showed that GIN1 localizes to microsomal membranes and more specifically to the cell membrane. Our hypothesis is that GIN1 undergoes splicing at the cell membrane induced by a plant sterol. This, together with its intrinsic ATPase activity, would help Gin1-N to interact with other proteins to control fungal cell growth and initiate the developmental switch to the symbiotic modus.

S48IS2 - 0690

Transcriptional responses of *Paxillus involutus* and *Betula pendula* during formation of ectomycorrhizal root tissue

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Ectomycorrhiza (ECM) are formed by mutualistic interactions between fungi and the roots of woody plants. During symbiosis the two organisms exchange carbon and nutrients in a specific tissue that is formed following the contact between a compatible fungus and plant. The ECM root tissue is characterized by distinct morphological and developmental stages, such as preinfection and adhesion, mantle and Hartig net formation. To gain some insights into the genes and pathways that are expressed during these stages, the transcriptional responses of *Paxillus involutus* and *Betula pendula* during formation of the ectomycorrhizal root tissue have been analyzed using cDNA microarrays. The basidiomycete *P. involutus* is a common fungus in temperate ecosystems and forms ECM with a number of different hosts including birch (*B. pendula*). The cDNA array contained cDNA probes obtained from a nonredundant set of expressed sequence tags (ESTs) produced from cDNA libraries of the ECM tissue, saprophytically grown mycelium or of axenically grown plant roots. Analysis of the array data showed that in comparison with nonsymbiotic conditions, 251 fungal (from a total of 1075) and 138 plant (1074 in total) genes were differentially regulated during the formation of the ECM tissue. During mantle and Hartig net development, there were several plant genes upregulated that are normally involved in defense responses during pathogenic fungal interactions. These responses were at later stages of ECM development, repressed. Other birch genes that show differential regulations involved several homologs that usually are implicated in water permeability (aquaporins) and water stress tolerance (dehydrins). Among fungal genes differentially upregulated during stages of mantle and Hartig net formation were homologs putatively involved in respiration. In fully developed ECM tissue, there was an upregulation of fungal genes related to protein synthesis and the cytoskeleton machinery.
The cDNAarray has also been used to examine the transcriptional responses that could be related to variation in host specificity in strains of *P. involutus*. Gene expression patterns were compared in three strains including Nau which is not compatible with birch and poplar, and the two compatible strains Maj and ATCC 200175. A set of 37 genes out of the 1075 fungal probes on the microarray were differentially expressed in the two compatible strains as compared to in the non-compatible strain following the contact with birch roots. An evolutionary analysis of these symbiotic regulated genes showed that two of them have evolved at an enhanced rate in Nau. These two genes displayed sequence similarities to a concanamycin-induced protein (cipC1) and a metallochaperone (cchA), respectively. The sequence divergence of *cipC1* and *cchA* can be explained by a decreased selection pressure and functional constraints of these proteins in Nau as compared to in the compatible strains.

S48IS3 - 0706

Acquisition and long distance translocation of phosphorus in the symbiotic phase of arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal (AM) fungi form symbiotic associations with the majority of land plants and improve the growth of host plant through enhanced uptake of phosphate (Pi). In the symbiotic phase, the fungi construct an extraradical hyphal network which has greater contact area with soil solution. The greater efficiency in Pi acquisition in the associations is not achieved only through the extension of hyphal network but also through the coordination of gene expression in the both symbionts, such as up-/down-regulation of the high-affinity Pi transporter genes in the fungus/host and up-regulation of the secreted acid phosphatase gene of the host. Pi taken up from the soil solution to the fungal cell is converted into inorganic polyphosphate (polyP), a linear polymer of Pi linked by high-energy bonds and a translocation form of Pi in the fungi. The synthesis and accumulation of polyP in the hyphae occur quite rapidly, but the biochemical and molecular processes are largely unknown. A bacterial enzyme, polyphosphate kinase, responsible for polyP synthesis has been well characterized: the enzyme synthesizes polyP using ATP as a phosphoryl donor. Recently, similar activity has been detected in the membrane fraction of AM fungi. It is suggested that polyP is accumulated in acidic compartments such as vacuoles. There are at least two types of acidic compartments in the fungi: one is tubular vacuole and another is spherical (rather small) vacuole. Although the involvement of these organelles in the polyP accumulation and translocation has not been clarified yet, biochemical and cytochemical approaches are being undergone.

S48PS1 - 0517

Pre-penetration apparatus: an arbuscular mycorrhiza-specific cell response in root epidermis

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Although very little is known about the nature of the molecular dialogue mediating partner recognition during the arbuscular mycorrhizal (AM) interaction, genetic studies on legumes (Pisum, Medicago, Lotus) have revealed that a small number of plant genes are essential for root penetration (Parniske, 2004).
Cytological studies have shown that, in the majority of cases, hyphae grow across the epidermal cell lumen surrounded by an apoplastic compartment and an invagination of the plant plasma membrane (Bonfante, 2001). This is a crucial checkpoint step in the plant/fungal interaction involving direct cell-to-cell contact and is probably essential in order to avoid defensive responses of the plant prior to root colonization (Novero et al., 2002).
Making use of GFP-labelling of intracellular components and a technique for targeted AM inoculation of in vitro transformed *M. truncatula* roots (Chabaud et al., 2002) we were able to visualize the assembly of a novel cytoskeletal/ER-containing apparatus in epidermal cells following hyphal contact and appressorium formation but prior to cell penetration. Our findings show that this so-called Pre-Penetration Apparatus (PPA) defines the path of subsequent hyphal infection and is most likely responsible for organizing the interface compartment (Genre et al., 2005). We have also shown that PPA assembly requires functional *DMI2* and *DMI3* genes. Mutation of these essential "endosymbiosis" genes blocks both rhizobial and AM infection. Finally, expression of MtENOD11 (an early nodulin gene expressed in both AM and rhizobium-colonized roots) has been detected specifically in epidermal cells prior to and during PPA development.
In striking contrast, contact with fungal pathogens such as *Phoma medicaginis* or *Colletotrichum* trifolii induces only a localized aggregation of cytoplasm and organelles, but no organization of PPA-like structures, thus underlining the specificity of the host root response to AM fungi. In conclusion, our studies show that the host plant plays an active role in preparing and directing AM fungal penetration across the root epidermis, thus providing a cellular explanation for the known genetic control exerted by plants in endosymbiotic associations.
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S48PS2 - 0023

Molecular identification of fungal endophytes in australian myco-heterotrophic orchids

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All orchids rely on fungal endophytes for seed germination and growth. Conservation of rare orchid species requires identification of these associated fungi, both for *ex situ* growth and reintroduction to the wild. The fungal endophytes of Australian myco-heterotrophic orchids have remained unidentified to date as they have been difficult to isolate into pure culture. In this study, direct PCR of colonised orchid roots, cloning and sequencing of fungal ITS regions have been used to identify the fungal endophytes of three species of Australian myco-heterotrophic orchid, namely *Dipodium variegatum*, *Dipodium hamiltonianum* and *Erythrorchis cassythoides*.
Genomic DNA was extracted from colonized roots of the three orchids from sites in Queensland and New South Wales, Australia. Fungal DNA was amplified with ITS1F and ITS4 primers and transformed into *E. coli*. Recombinant plasmids were isolated and fungal ITS inserts sequenced using the T7 primer. Sequences were compared with fungal ITS sequences in Genbank using BLAST searches.
The fungal community of *Dipodium variegatum* included *Russula*, *Verticillium* and *Trichoderma* spp. The fungal community of *Dipodium hamiltonianum* consisted of *Russula*, *Gymnomyces* and *Penicillium* spp. Analysis of the fungal endophytes of *Erythrorchis cassythoides* suggests that the orchid is colonized by both ectomycorrhizal fungi such as *Russula*, *Coltricia* and *Sebacina* as well as the saprotrophic *Gymnopus*.
These results suggest that like North American myco-heterotrophic orchids, Australian myco-heterotrophic orchids are commonly colonised by members of the Russulaceae. Conservation strategies for these orchids will require growth of these fungi under laboratory conditions and, as there are such techniques now available, this is a realistic proposition. As these fungi are ectomycorrhizal and the three orchid species typically grow at the base of *Eucalyptus*, the orchids may be indirect parasites on the trees but this remains to be proven. The involvement of *Gymnomyces* spp as endophytes in *D. hamiltonianum* may be one reason why this orchid is becoming rare as the fruit bodies of this species are much sort after food of fungivorous marsupials which may act as dispersal agents for fungal spores. The occurrence of both ectomycorrhizal and saprotrophic fungi as endophytes in *Erythrorchis* suggests that the orchid may be able to survive the death of its host tree by switching from a parasitic mode of nutrition to a saprophytic one.

1030-1230

SYMPOSIUM 49 - *Marine Fungi*

S49IS1 - 0121

Biodiversity of marine filamentous fungi and their phylogenetic relationships

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Although marine fungi and lichens were reported as early as the 1840's it was not until the studies of Sparrow (1937) on marine chytrids and Barghoorn and Linder (1944) on wood inhabiting marine ascomycetes that marine mycology took off. Early investigations focused on their documentation, description and taxonomy. Over the past 100 years progress has been remarkable with over 500 filamentous species documented, and new taxa are still being described. Early studies were based on morphological observations, but when this proved inadequate scanning and transmission electron microscopy was used to determine ascospore appendage ontogeny and this resolved a number of taxonomic problems. However, the advent of molecular techniques has resolved the phylogenetic relationships of many marine ascomycetes, especially in the orders Halosphaeriales and Lulworthiales. Different regions of rRNA gene, including SSU, ITS1-5.8S-ITS2 and LSU, have been sequenced. Our talk will focus on recent developments in resolving the phylogeny of the genera *Haligena, Halosarpheia, Halosphaeria, Lignincola, Marinospora, Remispora* and recently described genera: *Morakotiella, Pseudolignincola, Sablecola, Thalespora* (Halosphaeriales). We will focus on the question of stability of certain morphological characters within the Halosphaeriales when superimposed on to a phylogenetic tree. The stability of morphological characters in the classification of the Halosphaeriales will be discussed, for example ascospore appendage development types have evolved many times within the order and is not stable. The taxonomic position of *Swampomyces*, *Torpedospora* (Hypocreomycetidae *Incertae Sedis*, Sordariomycetes) remains unresolved at this time. Sequence data has also enabled the referral of a number of marine bitunicate ascomycetes to orders: *Aigialus, Julella, Paraliomyces* and *Verruculina.* The teleomorphs of selected marine anamorphic fungi have been confirmed: *Dendryphiella arenaria, D. salina, Orbimyces spectabilis, Sigmoidea luteola* and *Zalerion maritimum*.

S49IS2 - 0199

Documentation of marine fungal diversity: classical vs. molecular techniques

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Marine fungi comprise an ecological group of primarily filamentous ascomycetes, their anamorphs and yeasts. Traditionally, documentation of marine fungi involves collection of substrata from the environment, incubation of samples and identification of fruiting structures using microscopic techniques. This practice has been useful in recording over 510 filamentous obligate marine fungi. Morphological tools, however, fail to record fungi that do not sporulate in culture or on substrata. Culture-independent techniques, which are largely molecular, implement the shortcoming of classical techniques to accurately assess diversity as both fruiting and vegetative structures contain DNA. Molecular methods basically involve extraction of total environmental DNA, amplification of target genes using fungal-specific primers and separation of DNA fragments. Common techniques include denaturing (temperature) gradient gel electrophoresis (DGGE/TGGE), gene cloning, single-strand-conformation polymorphism (SSCP) and terminal restriction fragment length polymorphism (T-RFLP) for community profiling and/or identification of taxa in the community. A greater fungal diversity has been found in studies incorporating molecular techniques but both morphological and molecular approaches should be used to assess diversity accurately. A summary of classical and molecular tools for the documentation of marine fungi will be reviewed.

S49IS3 - 0058

Recognition of a Caribbean Marine Fungus as a New Genus by Classical and Molecular Characters

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Isolation of an unusual fungus from inter-tidal, mangrove-associated, sediment provided a source of novel secondary metabolites, including an aza-anthraquinone and caffeine (recognised for the first time as a natural fungal product), in static and shaken-culture fermentation. However, its intense black and apparently sterile mycelium confounded all attempts at identification. Through a persistent international European collaboration during 10 years, multi-cellular spores have at last been observed within cultures on very weak agar media, for example potato carrot agar made from fresh young vegetables, and nucleic acid sequences (ITS, and 18S and 28S rDNA) have been obtained. The latter together show, by reference to appropriate databases, that the fungus can not be placed in any current genus.
The present purpose is to report and illustrate the mycological, molecular, cultural and metabolite findings and show their putative influence on taxonomy of some other marine fungi. Characters that fit the fungus for the ecological nice in which it was found will be considered, and attempts at recognition of this and similar isolates in other tropical marine environments will be encouraged.
Particular interest in the fungus arose because of its metabolite profile that was highlighted in a multi-isolate screen for biosynthetic potential amongst new fungi isolated from a tropical marine environment. Micro-cultures were made in 200Ìl Czapek Dox broth enriched with yeast extract and fed with various radio-labelled primary precursors of secondary metabolism. Autoradiography of extracts resolved by TLC revealed metabolites derived from key precursors.
In submerged shaken fermentation the fungus grows as small, granular, spherical pellets. Agar cultures are robust in resisting desiccation.
The discovery of this fungus reiterates that marine exploration can still reveal novel biodiversity and to yield novel biosynthetic potential, the products of which are currently being explored further.

S49PS1 - 0027

Metabolic profiles support species concept of two marine *Dendryphiella* species: *D. arenaria* and *D. salina*

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Two species of *Dendryphiella* are known to be of marine origin, *D. arenaria* and *D. salina*. These species are usually identified based on differences in their spore morphology and colony appearance. We were interested in determining to what extent the species can be differentiated on the basis of their metabolic profiles. The *Dendryphiella* strains were isolated from various substrates collected along coastal areas in subtropical (Gulf of Mexico) and temperate (North Sea, Baltic Sea, Mediterranean Sea, English Channel) waters. Production of enzymes using cultural methods and API ZYM assay, as well as BIOLOG Phenotype Microarrays (PM) were used to assess the ability of *Dendryphiella* strains to utilize different substrata. Secondary metabolic profiles of the crude extracts of *Dendryphiella* strains grown on salted Malt Extract – Peptone – Yeast Extract agar medium were also determined. The *Dendryphiella* species from different geographical locations exhibited similar enzyme and secondary metabolic profiles, but differed significantly in their carbon utilization profiles. Not only the two species, but also two populations of *D. arenaria* could be differentiated with this PM.

S49PS2 - 0128

Morphological and molecular observations of *Manglicola guatemalensis*, a poorly known ascomycete

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Two collections of the poorly known ascomycete *Manglicola guatemalensis* from Trang and Trat (Koh Chang National Park) Provinces, Thailand, were made in 2005 on the palm *Nypa fruticans.* This fungus is only known from two previous collections (Kohlmeyer and Kohlmeyer, 1971; Hyde, 1988). This paper reports on the morphological characteristics and molecular phylogeny of this unique marine bitunicate ascomycete. *Manglicola guatemalensis* has large clavate to obtusely fusiform ascomata, wide ostiole surrounded by long hyaline hairs, bitunicate asci, deliquescing early to release, unequally one-septate ascospores, constricted at the septum, apical cell larger, chestnut-brown and a small light brown basal cell. Ascospores germinate readily, always from the basal smaller cell, which yield 8 isolates. Four strains (from the different locations) were selected for the phylogenetic study. Kohlmeyer and Kohlmeyer (1971) concluded that *M. guatemalensis* belonged in the Pleosporaceae / Venturiaceae, while Huhndorf (1992) erected a new family, the Hypsostromataceae, for the genera *Hypsostroma* and *Manglicola*. Different regions of the rRNA gene including SSU, ITS1-5.8S-ITS2 and LSU were sequenced. SSU sequences positioned *M. guatemalensis* in the Jahnulales clade, but with weak bootstrap support (58%) based on the maximum parsimony analysis. Common features with the Jahnulales include: stipitate ascomata, bitunicate asci, reticulate pseudo-paraphyses, bicelled brown ascospores. Ascospores of *M. guatemalensis* superficially resemble *Katumotoa bambusicola*, assigned by Harada *et al* (2005) to the Pleosporales. *Manglicola guatemalensis* differs from other bitunicate ascomycetes by large ascomata (1250 mm X 425-500 mm) wide ostiole surrounded by long hyaline, straight hairs, large unequally bicelled ascospores and its mangrove habitat growing on *Rhizophora mangle* and the frond base of *N. fruticans*. No clear phylogenetic resolution can be advanced for the ITS and LSU rDNA sequences, as few are available for phylogenetic comparison.

1030-1230

SYMPOSIUM 50 - *Mycetozoan Biodiversity*

S50IS1 - 0155

Global diversity of cellular slime molds

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In his 1984 monograph, Kenneth Raper listed approximately 50 described species of cellular slime molds (or dictyostelids) that were assigned to three genera (*Dictyostelium*, *Polysphondylium* and *Acytostelium*). In 1989, just a few years later, H. Hagiwara added a several more taxa to the group in his treatment of Japanese dictyostelids. Since then, the number of dictyostelid taxa described in the literature has doubled, and additional forms likely to represent new taxa are currently under analysis. The explanations for this expansion of dictyostelid biodiversity include such things as a greater intensity of sampling in survey efforts by a number of researchers, sampling of habitats and environments not previously surveyed, and an increasing body of evidence that some dictyostelid isolates previously assigned to a species as a variation of a type are now, by virtue of being represented by additional isolates, considered to deserve treatment as separate, distinct taxa. The utilization of molecular characters as well as morphological ones has also contributed to supporting an increase in apparent variation in the group. This report will attempt to summarize what is known about the biodiversity of dictyostelids throughout the world.

S50IS2 - 0142

A global perspective on myxomycete biodiversity

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The myxomycetes (also called plasmodial slime molds or myxogastrids) are the largest and best known of the eumycetozoans. Members of the group have been known from their fruiting bodies since at least the middle of the seventeenth century. There are approximately 875 recognized species of myxomycetes, many of which have been described in the past half century. The majority of species are probably cosmopolitan, but a few species seem to be confined to the tropics or subtropics and some others have been collected only in temperate regions of the world. Myxomycetes appear to be particularly abundant in temperate forests, but at least some species apparently occur in any terrestrial ecosystem with plants (and thus plant detritus) present. Field-based studies carried out in many different regions of the world over the past two decades have generated a considerable body of information that has provided evidence for a number of ecological patterns not reported previously for myxomycetes while also continuing to substantiate patterns or general observations that have long been suspected. Among the more important of these are that (1) temperature and moisture are the two most important factors determining the distribution and occurrence of myxomycetes in nature, (2) certain species of myxomycetes are invariably associated with particular microhabitats such coarse woody debris or the bark surface of living trees, (3) species-rich temperate deciduous forests are characterized by a level of myxomycete biodiversity that is as high or even higher than that of tropical forests, (4) in tropical forests, distinct assemblages of myxomycetes are associated with microhabitats such as aerial litter and the inflorescences of large herbaceous plants that have no counterparts in temperate forests, (5) the assemblage of myxomycetes found in deserts is more diverse than realized previously, and (6) in spite of the fact that the spores of myxomycetes would appear to have a high potential for dispersal, some species are much more common in one region of the world than another, even when climate and vegetation of the two regions being compared are fairly comparable. Although our knowledge of the biogeography, ecology and global distribution of myxomycetes has increased considerably, there is still a need for additional research.

S50IS3 - 0294

Global distribution of the protostelids with particular emphasis on the deep Southern Hemisphere

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An effort is under way to document the global biodiversity of the slime mold taxon *Eumycetozoa* (myxomycetes, dictyostelids, and protostelids). As part of this project, we have been collecting and cataloguing protostelids from many areas of the world that have not previously been assessed, examining old collection records, both published and unpublished, and including them in an interactive database that will allow researchers access to the global distribution of this poorly known group. Not only will the database provide distributional information on the taxa of protostelids, it will also link with identification aids such as keys and photomicrographs and with information on the present ideas of their classification and nomenclatural status. One goal of the project is to test the competing hypotheses about the global patterns of the distribution of eukaryotic microorganisms. One hypothesis suggests that because of their small size and the ease with which they could be transported, species should be found equally abundantly in any habitat where they occur. The alternate hypothesis is that ubiquity is not the case and that species will have distinctly different distribution patterns from each other. The fact that all but one of the described species of protostelids have been found in Hawaii, the most isolated archipelago on Earth, might be seen as evidence for the ubiquity hypothesis. However, when the South Island of New Zealand and Argentine Patagonia and Tierra del Fuego are compared, there are differences that suggest that similar habitats do not support similar protostelid biotas. When the southern regions are compared with Northern Hemisphere sites at similar latitudes, again there appears to be little support for the general hypothesis of ubiquity of species. We predict that further work will tend to weaken support for the ubiquity hypothesis, at least with respect to the protostelids.

S50PS1- 0146

Dictyostelid cellular slime molds of Australia

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Dictyostelid cellular slime molds associated with caves in the states of Alabama, Arkansas, Indiana, Missouri, New York, Oklahoma, South Carolina, Tennessee, West Virginia in the United States along with Puerto Rico and San Salvador in the Bahamas were investigated during the period of 1990–2005. Samples of soil material collected from more than 100 caves were examined using standard methods for isolating dictyostelids. At least 17 species were recovered, along with a number of isolates that could not be identified completely. Four cosmopolitan species (*Dictyostelium sphaerocephalum*, *D. mucoroides*, *D. giganteum* and *Polysphondylium violaceum*) and one species (*D. rosarium*) with a more restricted distribution were each recorded from >25 different caves, but three other species were present in >20 caves. The data generated from this study were supplemented with all known published and unpublished records of dictyostelids from caves in an effort to summarize what is known about their occurrence in this habitat. Based on these data, dictyostelids would seem to be consistently present in the assemblages of microorganisms found in caves, with 102 of the 123 (83%) caves known to have been examined for the presence of dictyostelids yielding at least one species. In West Virginia, the region for which the most data exist, dictyostelids were recovered from 95% of the 61 caves investigated. Most records of dictyostelids in caves are from temperate North America, but these organisms also were recovered from 11 of 13 (85%) caves surveyed in Puerto Rico and the Bahamas.

S50PS2 - 0144

Dictyostelid cellular slime molds from caves

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In his 1984 monograph, Kenneth Raper listed approximately 50 described species of cellular slime molds (or dictyostelids) that were assigned to three genera (*Dictyostelium*, *Polysphondylium* and *Acytostelium*). In 1989, just a few years later, H. Hagiwara added a several more taxa to the group in his treatment of Japanese dictyostelids. Since then, the number of dictyostelid taxa described in the literature has doubled, and additional forms likely to represent new taxa are currently under analysis. The explanations for this expansion of dictyostelid biodiversity include such things as a greater intensity of sampling in survey efforts by a number of researchers, sampling of habitats and environments not previously surveyed, and an increasing body of evidence that some dictyostelid isolates previously assigned to a species as a variation of a type are now, by virtue of being represented by additional isolates, considered to deserve treatment as separate, distinct taxa. The utilization of molecular characters as well as morphological ones has also contributed to supporting an increase in apparent variation in the group. This report will attempt to summarize what is known about the biodiversity of dictyostelids throughout the world.

POSTER ABSTRACTS S5

1330-1430
POSTER SESSION 5: BIODIVERSITY AND CONSERVATION

PS5-483-0024
Myxomycetes from Delta Region in Egypt
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The results of the second inventory of myxomycetes in Egypt are reported. The first was in Upper Egypt and the second is in North Egypt (Delta region). The substrates were wood, bark of living and dead tree and leaf litter. 26 species belonging to 19 genera of myxomycetes were identified. Wood was the best substrate for myxomycetes colonization. The most common species were *Tubifera ferruginosa*, *Physarella oblonga*, *Arcyria cinerea* and *Trichia favogina*. Brief description and classification of species are provided.

PS5-484-0029
Diversity of endophytic pestalotiopsis from podocarpaceae, theaceae and taxaceae in Southern China
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Diversity of endophytic fungi on a specific group of plants have been reported frequently and diversity of a specific endophytic fungi have been reported few. We conducted a survey on the diversity of endophytic *Pestalotiopsis* species in southern China from 2001 to 2003. In total we isolated 24 species of endophytic Pestalotiopsis from 10 plant species belonging to Podocarpaceae, Theaceae and Taxaceae, in Nanning, Guangxi province; Kunming, Yunnan province and Hangzhou, Zhejiang province. Species diversity of the endophytic Pestalotiopsis was illustrated at different levels. Among the species identified, 17, 16 and 4 species were obtained from Podocarpaceae, Theaceae and Taxaceae respectively; the Shannon-Wiener indexes in the three host families were 2.22, 2.28 and 1.06, respectively. Species diversity varied between individual host species, for example 15 *Pestalotiopsis* species were isolated from *Podocarpus macrophyllus*, 7 species from *Camellia sasanqua*, 5 species each from C. nitidissima and *Podocarpus* nagi, 4 species each from *Camellia japonica* and *C. sinensis;* and 2 species from each of *Camellia reticulate*, C. *oleifera*, *Podocarpus macrophyllus* var. maki, *Taxus yunnanensis* and *T. chinensis* var. *mairei*. Colonization frequencies of endophytic Pestalotiopsis species changed with host plants and their age and even organs. Generally the endophytes were more frequently obtained from twigs than from leaves of host plants. However, the colonization frequency of the endophytes was not proportional to the species diversity. Host recurrence of endophytic *Pestalotiopsis* varied with different species, *P. neglecta* and *P. photiniae* were isolated from plants in all the three families, but P. karstenii was isolated from only *Camellia japonica* and *C. sasanqua* of Theaceae. A phylogenetic tree was generated using ITS and 5.8S rDNA sequences of 44 *Pestalotiopsis* strains. Endophytic and pathogenic *Pestalotiopsis* strains from different host plants, and from different families, which were identified as the same *Pestalotiopsis* species always clustered together in a strongly supported branch. The present work supports the conclusion that *Pestalotiopsis* species are not generally specific to host plants.

PS5-485-0031
Identification of fungal associates of some deciduous tree species in Azerbaijan
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Natural forests in Azerbaijan are most commonly mixtures of deciduous plant species at elevations of 400-1600m. Diseases caused by fungi are thought to be major threats to these forest ecosystems. To assess the potential risk of disease to forest trees in Azerbaijan, we have begun to analyze the presence of pathogenic fungi and their impact on hardwood forests. Dutch elm disease has had considerable impact on elms (*Ulmus* spp.) in Azerbaijan during the last 50 years. The causal agent was thought to be *O. novo-ulmi*, but this has not been confirmed. Sweet chestnut (*Castanea sativa*), oaks (*Quercus spp.*) and walnut (Juglans regia) also have shown some signs of decline in the last few years. The aim of this study was to identify the most common fungal associates of these hosts. Fungi were isolated from both natural forests and plantations and characterized based on morphology and rDNA sequences. The identified fungi included *Armillaria* mellea and *Cryphonectria parasitica*, which represent new reports for Azerbaijan. The identified *Cryphonectria parasitica* is a serious pathogen of sweet chestnut. We have also confirmed that Dutch elm disease in Azerbaijan is caused by *O. novo-ulmi*. Other fungi identified are considered saprophytes or weak pathogens, including *Nectria haematococca*, Phoma pinodella, and *Sporothrix* sp.. Collection of more fungal isolates, especially agents that could be threats to the natural forests and plantations are planned.

PS5-486-0036

Mycotest for ecological evaluation of aquatic and terrestrial ecosystems

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The micromycetes as the essential part of aquatic and terrestrial ecosystems should be included in the list of biotic parameters for the ecological control. This proposal is based on experimental evidences by means of mycodiagnostics of the environmental quality. Earlier we have determined the list of the mycobiotic characteristics which can be recommended as rather informative parameters to discriminate the different zones (homeostasis, stress, resistance, repression) in some terrestrial and aquatic ecosystems. In spite of their variability some important mycological parameters (the number of the colony formation units, fungal biomass, species and interspecies biodiversity, morphological and physiological features of colony, rate of organic matter destruction etc.) use in soil and water bioindication. But the bioindication in situ is only one part of the biodiagnostics. It is necessary to prove the health of ecosystems with the result of laboratory biotests. There are several biotest-systems which are recommended for ecological control and standardization of natural and manmade media and substrates, but almost all of them are based on hydrobionts (*Daphnia magna, Paramecium caudatum, Scenedesmus quadricauda* etc. excluding tests for agricultural and fish-breeding purposes) and they are used for tests of water extracts from the samples. We suggest checking the toxicity in the laboratory experiment with the mycotest method on the spore germination of *Fusarium oxysporum*, which is enough sensitive to model toxicants during the first time (18-ours) of growing. The stable suppression of *Fusarium oxysporum* conidia germination (LD50) was observed already by concentration 0.5 ppm K2Cr2O7 (we use it as a toxicant of comparison). This fact and other features of this test-organism (i.e. fast-growing colony, thin-walls conidia, simple preparing of spores suspension etc.) allow us to recommend this method for ecological control of toxicity of the aquatic and terrestrial ecosystems. As pedobiont *Fusarium oxysporum* can reflect not only the ecotoxicity of the soluble components in water extracts, but also the solid phase of soils and substrates.

PS5-487-0040

Diversity and host specificity of leaf-inhabiting endophytic fungi in a temperate deciduous forest canopy

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The diversity and ecology of fungal endophytes in leaves of trees can be considered as scarcely studied, as only a few wooden plants fulfill economic criteria enough after which studies of endophytes are planned more often than not. Endophytic diversity and patterns of occurrences is widely unknown despite the knowledge that endophytes influence organismical interactions in many ways.

We used the Leipzig Canopy Crane research facility to sample leaves of different tree species in a temperate, mixed deciduous forest canopy between 10-30 m in height for the occurrence of endophytic fungi in spring and autumn 2005. After surface sterilisation, the leaves were cut into small fragments which were placed on Malt Extract Agar. Emerging colonies were isolated. By means of morphology and DNA sequencing they were identified at generic or species level.

The number of colony forming units (CFU) from the spring samples was lower with 216 fungi than that of autumn leaves (417 CFU). Preliminary results revealed the abundant and widespread fungi *Aureobasidium pullulans* (teleomorph *Discosphaerina fagi*), *Alternaria* sp., *Aspergillus* spp., and *Cladosporium* spp. as beeing endophyic in young spring leaves. Interestingly, genera such as *Xylaria, Phoma*, and *Valsa*, identified as fruit body-forming lignicolous fungi in previous studies in the canopy at the same crane site also could be isolated from various leaves. This indicates that leaf-endophytic fungi probably follow the source-sink directions from inside the wood into the tree's buddings.

Analyses are ongoing to depict patterns of occurrence in different host trees, in sun and shaded leaves, and in the upper and lower canopy layer.

The initial studies could be regarded as a starting point to bringing more light into the diversity and ecology of endophytic fungi in temperate deciduous forests and how they affect organismical interactions.

PS5-488-0060

Ecology of the rare tooth fungi *Hericium cirrhatum*, *H. coralloides* and *H. erinaceus* in Britain

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Hericium coralloides, H. cirrhatum, H. erinaceum are on the provisional Red Data List of British Fungi. H. erinaceum is also protected by UK law. These rare wood-decay basidiomycetes are found predominantly in southern England where they grow mainly on beech in ancient woodland. Little is known of their ecology. They are readily cultured in vivo, and H. erinaceum is cultivated for food in several countries. Why are they so scarce in the wild? Arrival and establishment in a suitable substrate and subsequent growth and competition within that resource are likely to be key to their rarity. These questions were investigated using spore trapping, molecular methods to locate species within wood, and competition between the target species and likely competitors.
To assess spore travel from a sporulating fruit body, homokaryotic mycelium on agar was used as bait. Dishes were placed 0-10m from the fruit body for 4-24 hours. Heterokaryotisation was determined by presence or absence of clamps. Over 99% of homokaryotic cultures were heterokaryotised. Spore trapping methods were successful, and refined methods will be used in future to determine the extent of aerial dispersal.
Primers that distinguished *H. cirrhatum* (HER2F/HER3R) from other species, and primers which distinguished *H. coralloides* and *H. erinaceum* (HER2F/HER2R) from other species, were developed. Logs in which *H. cirrhatum* had been previously inoculated were sampled and analysed using molecular methods and traditional isolation from wood onto agar. Molecular methods often confirmed suspected presence of H. cirrhatum where isolation from wood onto agar failed. Molecular methods provide an invaluable tool for assessing presence of fungi in the mycelial form – sampling from likely sites could show if the species really are rare, or if it is just the fruit bodies that are scarce. The approach will also be used to determine whether or not these fungi are latently present within functional sapwood.
H. coralloides, *H. cirrhatum* and *H. erinaceum* were paired against 20 other wood decay species from beech on malt extract agar at 20°C, and against 17 species in wood blocks. All three species achieved deadlock or replaced over half the competitors. Results from agar and wood were similar. These fungi are, therefore, relatively combative, and this seems unlikely to be a factor resulting in their rarity.

PS5-489-0061

Aphyllophorales (Basidiomycota) from the Floresta Nacional de Caxiuanã, State of Pará, Brazilian Amazonia – preliminary results

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Aphyllophoraceous fungi are considered the major wood decomposers and play crucial role in nutrient cycling in arboreous and shrubby ecosystems. They are an artificial group of 23 families, the most important being Polyporaceae, Corticiaceae and Hymenochaetaceae. Their diversity is expected to be high in high-diversity ecosystems but since few researches have been undertaken in the Brazilian Amazonia, few Aphyllophorales have been registered until now.
The Floresta Nacional de Caxiuanã (National Forest of Caxiuanã) is a 330.00ha reserve managed by the Brazilian Institute of the Environment and Renewable Resources (IBAMA) and is located in the Amazonian State of Pará. Part of its area (33.000ha) is a scientific station (Ferreira Penna Scientific Station - ECFPn), 400km far from Belém, the capital of Pará. It has been about 15 years the ECFPn is being inventoried but still few Aphyllophorales have been reported. In the reserve, the typical Amazonian ecosystems can be found and its flora is reported as being one of the richer and denser in the Amazonian basin.
This research was based on material deposed at the herbarium (MG) of the Museu Paraense Emílio Goeldi, in Belém, and in one recent field trip undertaken on January 2006 to the ECFPn. More 3 field trips are expected to have place in the reserve and material previously deposed at MG is still being identified, which expect us to have increased the list below and improved the knowledge about the diversity of Aphyllophorales in Amazonia. The following Aphyllophorales have been identified until now: **Amauroderma exile, *A. partitum, A. praetervisum, A. sprucei, Ganoderma australe, G. stipitatum (Ganodermataceae); *Hymenochaete damicornis, *H. leonina, H. luteo-badia, Phellinus baccharidis, *P. calcitratus, P. extensus, P. fastuosus, P. gilvus, *P. shaferi, *P. undulatus, *Phylloporia spathulata (Hymenochaetaceae); *Scytinostroma duriusculum, S. portentosum (Lachnocladiaceae); Earliella scabrosa, Hexagonia papyracea, Lentinus crinitus, *Perenniporia martiusii, Polyporus dictyopus, P. guianensis, P. leprieurii, P. tenuiculus, Pycnoporus sanguineus, Rigidoporus microporus, *Rigidoporus biokoensis, Trametes modesta, Trichaptum sector (Polyporaceae); Schizophyllum commune (Schizophyllaceae); Caripia montagnei, *Cotylidia auratiaca, *Cymatoderma caperatum, C. dendriticum, Inflatostereum glabrum, Stereum ostrea (stereoid fungi). Species marked with an * are new records for the State of Pará.*

PS5-490-0079

Xylariaceous fungi in Doi Suthep-Pui National Park, Thailand

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Doi Suthep-Pui National Park located in the NorthernThailand, covers an area of 2,625 square kilometers. Doi Suthep, Doi Buakha and Doi Pui are the three main peaks in the park. The highest peak, Doi Pui, rises to 1,685 meters above mean sea levels. Because of the high altitude, the weather on the upper slopes of the mountains is cool and pleasant all year even in hot season. There are two basic types of forest on the mountain: Deciduous forest below about 1,000 m elevation and evergreen forest above. The deciduous is further divided into two kinds, deciduous dipterocarp-oak Forest in the driest areas and mixed evergreen deciduous forest along streams and gullies. Common species of trees are the families Dipterocarpaceae, Fagaceae and Magnoliaceae. This study is aimed to investigate the diversity and distribution of xylariaceous fungi in Doi Suthep-Pui National Park. The fungi can be broadly grouped according to their lifestyles into three groups phytopathogens, saprotrophs, and endophytes, and also have an excellent track record for the production of secondary metabolites, many of which have proved to be novel.

The field survey for investigating the xylariaceous fungi was carried out in San Ku, Monthathan Waterfall, and the trial to Mong Village in Doi Suthep-Pui National Park in 2005. Fungal specimens were collected, then identified and classified according to their macroscopic and microscopic characteristics of teleomorph stage and cultural characteristics.

Interesting data on species diversity and distribution of xylariaceous fungi were found in deciduous dipterocarp-oak Forest and mixed evergreen deciduous forest in Doi Suthep-Pui National Park in the Northern Thailand. These fungi were wood inhabitants, and collected from a variety of their habitats, particularly on logs, fallen branches and bamboo. A total of 25 taxa belonging to 6 genera: *Xylaria, Hypoxylon, Nemania, Biscogniauxia, Jumillera* and *Astrocystis* were recorded. Thirteen taxa could be identified. Twelve taxa have not been named yet, and are likely to be new. *Hypoxylon* is numerically the most important genus, and also exhibited the greatest distribution.

From this investigation, *Hypoxylon leptascum* and *H. leptascum var. macrosporum* are recorded a second time in Thailand. The first record is in Phu Hin Rongkra National Park in the Northern. These fungi have been found in the high area more than1,200 meters above the sea level. This study revealed the diversity of xylariaceous fungi found in Doi Suthep-Pui National Park.

PS5-491-0083

Filamentous fungi from coastal Arctic environment

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The Arctic and Antarctic regions have been investigated over the last decade mainly for the presence of psychrophilic Bacteria, Archaea and occasionally for algae. Rare scientific studies have shown as well sporadic presence of different fungi in permafrost layers, polar soil, vegetation, water, snow, and glacier ice. In all cases fungi were isolated using non-selective, "mesophilic" media.

In our study of fungal frequency and diversity in coastal Arctic environment, we have used different isolation media with lowered water activity, due to high NaCl or sugar concentrations, and low incubation temperatures. Isolates were identified using morphological characteristics and secondary metabolite profiles as detected by TLC and HPLC.

We have discovered high CFU numbers of filamentous fungi and melanized and non-melanized yeasts in different niches in the coastal Arctic environment. The CFU ranged from 1000-3000 l-1 in seawater, 6000-7000 l-1 in melted sea ice and up to 13.000 l-1 in melted glacial ice. The prevailing genus of isolated filamentous fungi was *Penicillium* with 32 identified species, followed by *Eurotium* with six and *Aspergillus* with four.

Ice formation results in decrease of biologically available water, thus water activity (aw) is the dominant external factor influencing microbial activity in extremely cold environments. Preventing the osmotic stress we were able to isolate high CFU numbers of filamentous fungi from Arctic seawater, sea ice, snow in the tidal range, and glacial ice.

Studies of psychro-tolerant and xero-tolerant fungi are important, since they enables on one hand the unraveling of biodiversity in natural environments, delimited by strong physicochemical interactions, and on the other hand give an insight into the natural occurrence of fungi, primarily known as contaminants of food, preserved at low temperatures.

PS5-492-0096

Endophytic Species Of Neotyphodium On Some Gramineous Species In Iran

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Some members of the family Clavicipitaceae are endophytic and have mutualistic relationship with the plant family Poaceae. Their relationship is beneficial for both host and fungus. In the present investigation, endophytic fungi were isolated from seed and leaf sheath of hosts Festuca arundinacea, *Festuca ovina, Festuca pratensis, Bromus tomentellus, Melica persica* and *Lolium prenne*. Morphological charecters were checked on different culture media. Most of the isolates obtained from wild barley belonged to section Albo-lanosa of the genus *Acremonium* and *Neotyphodium*. Sensitivity of the isolates to benomyl was tested and it was found that media containing benomyl enhanced sporulation of non-sporulating isolates. In antibiosis test, it was found that most of isolates were effective against the phytopathogenic fungus *Bipolaris australiensis* and only two isolates of FoGn and MpFn were effective against *Pythium aphanidermatum*. Based on morphological characters, the genus *Neotyphodium* on F. *arundinaceae*, F. *pratensis*, F. *ovina*, B. *tomentellus*, M. *persica* and L. *prenne* and the N. *coenophialum* on F. *arundinacea* and N. *festucae* on F. *ovina* and N. *lolii* on L. *prenne* and N. cf. *bromicola* on B. *tomentellus* were identified and this is the first report of these fungi from Iran.

PS5-493-0110

Changes in microfungal communities during decomposition of leaf litter

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Changes in microfungal communities were studied on decomposing leaf litter of dogwood *(Swida controversa)* and beech *(Fagus crenata)* using a litterbag method in a cool temperate forest in Japan. The two litter species differed in chemical quality and consequently in mass loss rate: dogwood had low lignin content (20% w/w) and lost more than 90% of its original mass during 18 months of decomposition, whereas beech had high lignin content (44% w/w) and lost only 20% of its original mass during that period. The purpose of this study was to examine how the difference in litter quality affected the pattern of microfungal succession. A total of 57 and 56 microfungal species were recorded during decomposition of dogwood and beech leaves, respectively, and species rank-frequency curves of communities were relatively similar between dogwood and beech. The number of species reached a maximum level after 9-11 months of decomposition for both litter species, but the maximum number of species was greater for dogwood than for beech. Cluster analysis indicated five successional groups on dogwood and three successional groups on beech; the groups on each litter species differed in colonization time during decomposition. Similarity (Pianka's similarity index) of the species composition between sampling occasions indicated that the microfungal community on dogwood continued to change throughout the 18 months, whereas the microfungal community on beech remained relatively constant. Niche analysis indicated that niche breadth and niche overlap of main microfungal species in terms of colonization time were greater on dogwood and than on beech. The higher litter quality in dogwood probably contributed to the coexistence of more microfungal species, a more rapid changes in species composition, and greater species packing in the community than in beech. This difference in the pattern of microfungal succession was possibly associated with the faster decomposition of dogwood leaf litter.

PS5-495-0122

Fungal succession on fallen leaves of an evergreen oak in Japan

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Fungal successions have been studied on fallen leaves of conifers and deciduous broadleaf trees in the temperate and sub-arctic zones in the northern hemisphere. In Japan, these on fallen leaves of pines, firs and beeches have also been studied. However, we have not had enough information on the fungal succession on fallen leaves of evergreen oaks that had covered the southern part of Japan extensively, though their forests now are scattered.
To study the fungal succession associated with the decay of an evergreen oak leaves, we tried to examine fungal communities on the leaves at different decomposition stages. An evergreen oak, *Quercus myrsinaefolia* was chosen for it is a dominant evergreen oak in the northern evergreen broadleaf forest in Japan.
The study site was selected in Meiji Shrine Forest located in central Tokyo (35°40'N, 139°41'E). The living and fallen leaves of *Q. myrsinaefolia* were collected at each season (four times). Collected fallen leaves were divided into three decomposition stages based on their colour and texture. Ten typical leaves were chosen from each stage, and two disks per one leaf were obtained with a 7mm cork borer (20 disks in total for one stage). The disks were washed with sterilized surfactant solution, then with water in series. These were cultured on corn meal agar plates, and observed regularly with a light microscope. Frequencies and constancies of individual fungi were calculated for each decomposition stage.
Results and discussion Clear differences were not observed in the species composition of high frequency and high constancy fungi among three decay stages, but their frequency values were fluctuated with the decay stage or the season. Based on the fluctuation patterns of frequency values of these species, we could recognize the following successional pattern. Phyllosphere fungi such as *Tubakia* sp. and Colletotrichum gloeosporioides colonized mature leaves in the canopy. Most of them disappeared quickly from freshly fallen leaves and some litter inhabiting hyphomycetes such as *Subramaniomyces fusisaprophyticus* and *Rhinocladiella intermedia* rapidly colonized such leaves and continued high frequency until the later stage of decay. On the contrary, some hyphomycetes such as *Chaetopsina fulva* and *Chaetospermum camelliae* appeared at high frequency on freshly fallen leaves at limited seasons. Finally soil fungi represented by some mucoralean species, *Gongronella butleri* and *Backusella circina* occurred mainly on heavily decayed leaves.

PS5-496-0123

Wood-fungi diversity, dead wood relations and habitat management in oak-dominated forest

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To reduce the serious taxonomic bias in conservation research, fungi should be given more attention. Most studies of wood-fungi have concerned polypores in boreal forests. Our results concern ascomycetes and basidiomycetes in the more diverse temperate broadleaved oak-dominated forest, one of the most heavily degraded biomes in the world. Diversity was studied in relation to 1) type and amount of dead wood in semi-natural stands, and 2) forest management. Twenty-five stands in southern Sweden were surveyed and 103 ascomycetes and 425 basidiomycetes were found.
Fungi on logs have been studied repeatedly, but dead wood also occurs on living trees, in stumps, and in fallen branches. We studied attached, standing (including stumps) and downed dead wood with a diameter exceeding 1 cm. The sites contained on average 14.3 m3/ha coarse woody debris (CWD; diameter >10 cm). Fine woody debris (FWD; diameter 1–10 cm) made up another 12.2 m3/ha. Total dead wood volume was dominated by downed (66%) and standing dead wood (22%), while attached dead wood and stumps amounted to smaller fractions (6% each).
Species density was higher for FWD than for CWD for both ascomycetes and basidiomycetes, but species richness was higher on CWD than FWD for basidiomycetes. Of the ascomycetes, 75% were found exclusively on FWD. Thus, FWD is important for diversity of wood-inhabiting fungi in this forest type, but CWD must also be provided for many species of basidiomycetes. Stumps of oak supported some rare species, e.g. the red-listed polypore Perenniporia medulla-panis. Attached dead oak branches supported a specialised fungal flora, e.g. the red-listed polypore *Pachykytospora tuberculosa* and the new ascomycete *Moristroma quercinum*.
Partial cutting which restores a semi-open forest structure has been proposed as a management method for biodiversity in forests with big oaks, and for forestry (e.g. biofuel production). To test the effects on fungal diversity, 25-30% of the basal area was harvested. Species richness of basidiomycetes declined more in experimental plots than in reference plots, but no effect was found for ascomycetes. Species richness on FWD was reduced, but we found no effect on CWD. Species composition did not change as a result of partial cutting, but the number of Red List species decreased from ten to four. The high biodiversity and sensitivity to forest management in wood-fungi, should be taken into account in management decisions concerning oak-dominated woodland.

PS5-497-0133

Endophytes of a peat swamp palm: *Licuala longecalycata*

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Studies are in progress to document saprobic and endophytic fungi of several palms growing in a peat swamp forest in Narathiwat southern Thailand. Senescent palm material (leaves, petioles, rachides) was collected from three habitats (aerial, on ground surface, submerged) in the peat swamp and the saprobic fungi documented and isolated. For endophytic fungi, discs approximately 5 mm were cut from healthy leaf tissue, surface-sterilized and plated on artificial media. One hundred and sixty eight saprobic fungi were recorded, with Annulatascus velatispora, *Diaporthe setulae, Massarina bipolaris, Microthyrium* sp., and *Phaeoisaria clematidis* as the most common species. One hundred and forty seven endophytic strains were characterised with xylariaceous fungi the dominant group (15% of the total with 22 strains). Only 3 saprobic xylariaceous fungi were recorded (Anthostomella, Astrocystis, Stilbohypoxylon) while no *Xylaria* species has been collected on any of the peat swamp palm samples investigated over a three year period. Isolation of endophytes from *Licuala spinosa*, collected at Kuan Kang forest, Trang Province, yielded 1289 strains characterised as 197 morphotypes of which 75 were xylariaceous. The question arises, why are Xylaria species so common as endophytes, yet none have been collected on decaying palm material? Sequence analyses from 28S and ITS were analysed phylogenetically using Maximum Parsimony (MP) and Markov Chain Monte Carlo (MCMC) analysis (Mr Bayes). Preliminary Phylogenetic results show that strain BCC16517 clustered in the Clavicipitaceae; strain BCC16504 is related to a *Hypoxylon* sp., strain BCC13183 groups with Astrocystis, Nemania and Rosellinia. Sterile *Xylaria* strains currently producing sterile stromata are being induced to sporulate on palm material under laboratory conditions. The results are discussed in relation to observations made by other researchers.

PS5-498-0139

Non-target impacts of an introduced *Trichoderma* biocontrol agent on soil microbes

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Fungal biocontrol agents (BCA), such as *Trichoderma* species, are used to control many plant diseases yet may pose risks to native non-target species. For example, non-target effects including mycoparasitism of beneficial soil fungi such as mycorrhizae, reduction in plant root colonisation by mycorrhizal fungi, disorders in commercial mushrooms, plant nodulation by *Rhizobium* spp., and changes in plant growth have all been associated with fungal biological control agents such as *Trichoderma* spp. This study is using *Trichoderma* as a model system to investigate non-target impacts of fungal BCA's on natural microbial populations to provide government authorities with information regarding the risks associated with BCA introductions. This information will then be used to guide the development of best-practice protocols for further BCA introductions in New Zealand. Non-target impacts of Trichoderma BCAs are being measured in a number of ways in this study. Firstly, changes to soil microbial communities following BCA introduction are being determined using Denaturing Gradient Gel Electrophoresis (DGGE). Particular emphasis was placed on impact on the diversity of the natural populations of Trichoderma species. To do this, *Trichoderma* genus-specific primers were designed targeting the ITS1 and ITS2 rDNA sequence regions. As the primers needed to be degenerate in nature, the PCR products produced were not suitable for direct DGGE analysis and so a semi-nested product that only included the ITS1 region was analysed. DGGE conditions are being developed to separate out the species known to exist in New Zealand soils. The technique will be employed to monitor changes in species diversity over time following the introduction of a commercial Trichoderma BCA preparation into the soil to determine the scale of the impact and if the native populations recover over time. Secondly, BCA impact on populations of mycorrhizal fungi are being monitored using well-established mycorrhizal indices, and finally the interaction between BCA cultures and beneficial microbes is being measured using challenge inoculations in the laboratory.

PS5-500-0145

Distribution and ecology of dictyostelids in the Great Smoky Mountains National Park (eastern North America)

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The Great Smoky Mountains National Park encompasses an area of 2080 km2 in eastern Tennessee and western North Carolina between 35° 28' and 35° 47' N latitude. Elevations range from approximately 270 to 2000 m above sea level, and the topography and vegetation are as diverse as any region of eastern North America. During the period of 1998 to 2004, soil/litter samples for isolation of dictyostelid cellular slime molds were collected throughout the Park. Collecting sites included examples of all major forest types along with the more common types of non-forest vegetation. More than 2300 clones of dictyostelids were recovered from 412 samples. These clones included representatives of 20 described species together with at least 10 species new to science. This total is higher than those reported for other temperate regions of the world. In general, both numbers of species and numbers of clones/g of sample material decreased with increasing elevation, and several species displayed a distinct preference for either the low or high end of the elevation gradient. The relatively high number of new species recovered from samples collected at high elevations is an important new finding for dictyostelid ecology and distribution.

PS5-501-0146

Dictyostelid cellular slime molds of Australia

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The continent of Australia, with a total extent of approximately 7,682,300 km2, covers about 5% of the earth's land area. Most of the continent is low, flat and dry; deserts and dry grasslands are the predominant vegetation types. During the 2001 to 2004 field seasons, samples for isolation of dictyostelid cellular slime molds were collected at a number of localities in Queensland, the Northern Territory, Western Australia, New South Wales and Victoria. The majority of these samples were collected form the soil/litter layer on the ground, but some additional samples were obtained from the layer of organic matter ("canopy soil") associated with the bases of vascular epiphytes on the trunks and branches of trees in the tropical forests of northern Queensland. Some of these samples were collected at heights of more than 20 meters about the forest floor. Many of the forms recovered from these samples could be assigned to described taxa, including such cosmopolitan species as *Dictyostelium mucoroides*, *Polysphondylium pallidum*, P. *violaceum*, and D. *giganteum*. However, a significant number of others appear to represent species new to science. The large number of apparently undescribed forms suggests that the dictyostelid biota of Australia is relatively distinct when compared to that of any other continent.

PS5-502-0150

Pathogenic Wood-decaying Fungi in China

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Pathogenic wood-decaying fungi in China were surveyed during last 10 years, and the wood destroying species were in particular investigated. 90 pathogenic Basidiomycetes were found in natural forests, forest plantations and garden forests, and among them 30 species were recorded for the first time on living trees from China. Among them *Fomitiporia bananaensis* Y.C. Dai, F. tibetica Y.C. Dai & M. Zang, *F. torreyae* Y.C. Dai & B.K. Cui, I*nonotus compositus* H. C. Wang, *Phellinus himalayensis* Y.C. Dai, Polyporus subvarius C.J. Yu & Y.C. Dai were recently found from China. Most of these wood-destroying fungi are polypores in the Aphyllophorales, and the majority were found in temperate and boreal forests. Although most polypores are restricted to particular species or genera of trees, some polypores can attack many hosts, e.g., *Ganoderma pseudoferreum* lives on over 20 trees of different angiosperms, and *Fomitopsis pinicola* occurs on many gymnosperm and angiosperm trees. 80 species cause a white rot, and 10 cause a brown rot. White rot species are distributed in almost all the Chinese forests, and they infect trees of both conifer and hardwoods. However, brown rot species occur primarily on coniferous trees, and they were found mostly in temperate and boreal forests. Limited investigations were made in southern China and only 18 species were found in the subtropical forests of China. Since tree species diversity is greater in southern China, it is likely that greater numbers of wood-decaying fungi would be found in this area. Additional surveys and basidiocarp identification is needed in this arera of China to obtain a more precise assessment of the major wood decay fungi that may be found.

PS5-503-0157

Fungi on Nypa fruticans - a mangrove palm

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In the present paper the fungi occurring on mangrove palm - *Nypa fruticans* are discussed. Totally 90 filamentous fungi occur on *N. fruticans* including 69 ascomycetes and 20 anamorphic fungi in addition to a microscopic basidiomycete *(Halocyphina villosa)*. *N. fruticans* can be considered as an amphibious palm that can grow both in saline and fresh waters but always near the coasts. We have investigated the mycota occurring on *N. fruticans* in Brunei and found that both marine and terrestrial fungi occur along the salinity gradient up to freshwater areas. However, the number of marine fungi seems to be more than the terrestrial fungi. *Aniptodera, Linocarpon* and *Astrosphaeriella* are the most common genera on this palm. While the former genus is a typical aquatic fungus the latter two commonly occur on other palms. *Linocarpon appendiculatum*, *L. bipolaris*, *Neolinocarpon globosicarpa* and *Oxydothis nypae* frequently occur on fronds, while Linocarpon bipolaris, Astrosphaeriella striatispora, Trichocladium nypae and Linocarpon appendiculatum occur more frequently on leaves. Some novelties on this host are members belonging to Annulatascaceae that have been reported recently. Two different studies on the vertical distribution of fungi on this host showed that the diversity is rich in submerged parts followed by intertidal parts when compared to aerial parts. This is in contrast to *Rhizophora* where diversity was more on the intertidal parts than completely submerged parts. Although most of the work done on this host has been from South East Asia, several mangrove formations within old tropics are yet to be investigated. *N. fruticans* is a threatened species and is lost in several hitherto known mangrove formations. The fact that 90 fungal species occur on this host of which more than 40 are host specific has relevance to global estimates of fungi and ecological significance. These aspects will also be discussed.

PS5-504-0158

Contribution to the knowledge of the biodiversity of wood-inhabiting Aphyllophorales (Basidiomycetes) in the Caucasus hotspot

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Wood-inhabiting basidiomycetes play an outstanding role in nature, considering the fact that they are the main organisms with the capability of decomposing wood to its primary constituents. Little is known about their occurrence in the Caucasus. This area encompasses the countries Azerbaijan, Armenia, Georgia, part of SW Russia, NE Turkey and part of NW Iran and is one of the most diverse regions in the world in the respect to both fauna and flora; and is regarded as a remarkable collection of many relict and endangered species. Some recent reports on the Biodiversity of Caucasus hotspot have compiled valuable data on the biodiversity of plant and animal communities. There have also been sparse studies on macrofungi in different parts of this region, but there is still no comprehensive study or even preliminary estimation about the total number of wood decaying basidiomycetes in the Caucasus as a whole. Consequently this study aims to first, introduce our ongoing survey on this group of fungi in the Caucasus area and then, summarizes the results of previous studies in the region, with special view on its Iranian part. There have been several works on wood inhabiting fungi in the North Iran in the Hyrcanian province and a recent study is going on in Arasbaran forests, NW Iran.
According to this survey, it was shown that altogether a total number of about 600 wood-decaying Aphyllophorales have been recorded in the Caucasus region. Some ecological notes on these fungi are presented together with notes on their distribution in the Caucasus

PS5-505-0161

Collection of wild edible fungi in Nepal

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A total of 235 species of wild fungi are confirmed to be used for food in Nepal. The collection is very frequent between 2000-4000m, where the vegetation is dominated by conifers and almost all households in these areas collect. In the lower part of the country collection of wild fungi is also common but confined to certain ethnic groups.
National forest and Community forest are the most frequently visited places for collection of wild fungi, whereas private land is less important.
The ethnic groups Thakalis, Sherpas, Tamangs and Newars are found to be relatively more dedicated to the collection of wild edible mushrooms than Brahmins and Chhetris. However, traditions are changing and often all ethnic groups in an area collect.
Wood inhabiting fungi such as *Latioporus sulphureus*, *Griffola frondosa* and *Lentinus* spp. are among the favourites in most areas. Mycorrhizal fungi like *Lactarius spp.*, *Russula spp.*, *Amanita* spp., *Suillus* spp. and *Scleroderma* spp. are frequently collected in temperate and subtropical pine forest and *Russula* spp., *Cantharellus* spp., *Boletus* spp. and *Laccaria* spp. are most frequently collected in subtropical broadleaved forest. Termitomyces spp. living in symbiosis with termites are very often collected in subtropical areas of the country.
Poisoning caused by mushroom is common and it is estimated that at least 15-30 persons die every year. Most of the cases seem to be caused by poisonous species of Amanita.

PS5-506-0163

Aphyllophorales (Basidiomycota) from the Reserva Biológica do Lago Piratuba, State of Amapá, Brazilian Amazonia – preliminary results.

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Aphyllophoraceous fungi are considered the major wood decomposers and play crucial role in nutrient cycling in arboreous and shrubby ecosystems. They are an artificial group of 23 families, the most important being Polyporaceae, Corticiaceae and Hymenochaetaceae. Their diversity is expected to be high in high-diversity ecosystems but since few researches have been undertaken in the Brazilian Amazonia, few Aphyllophorales have been registered until now.
The Reserva Biológica do Lago Piratuba (Biological Reserve of Piratuba Lake – Rebio Lago Piratuba) is a 395.000ha reserve managed by the Brazilian Institute of the Environment and Renewable Resources (IBAMA) and is located in the Amazonian State of Amapá (49°40' - 50°30'W, 1°50' - 1°27'N), 200km far from Macapá, the capital of Amapá. In this reserve, mangroves, periodically flooded prairies and tropical forest can be found.
The field trips to Rebio Lago Piratuba was undertaken on April/May and August 2004 and the following Aphyllophorales have been identified until now: *Ganoderma australe* (previously reported as *G. applanatum*), *G. stipitatum* (previously reported as G. lucidum) (Ganodermataceae); **Cyclomyces iodinus*, **Hymenochaete luteo-badia, *Phellinus calcitratus, P. gilvus* (Hymenochaetaceae); **Coriolopsis rigida, Earliella scabrosa, *Fomes fasciatus, Fomitopsis nivosa* (previously reported as *Tyromyces chioneus*), *Gloeophyllum striatum, Hexagonia hydnoides, *H. papyracea, Lentinus crinitus, *Polyporus guianensis, *P. leprieurii, *P. tenuiculus, *P. tricholoma, Pycnoporus sanguineus, *Trametes modesta, Trichaptum byssogenum, *T. sector (Polyporaceae); Schizophyllum commune (Schizophyllaceae); *Caripia montagnei, Cymatoderma dendriticum, *Lopharia cinerascens* (stereoid fungi). Species marked with an * are new records for the State of Amapá.

PS5-507-0169

Does Abandonment of a Wooded Meadow Affect Diversity and Community Structure of Ectomycorrhizal Fungi?

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Wooded meadows are seminatural, regularly mown, plant species rich ecosystems, which have declined >100-fold in North Europe, naturally developing into thickets. Ectomycorrhizal fungi provide mineral nutrition to dominant trees in wooded meadows. We hypothesized that abandonment of a wooded meadow affects below ground communities and diversity of ectomycorrhizal fungi. Sequencing of root tips and extrapolation were used to identify the fungi and to compare biodiversity patterns. We distinguished 172 species of ectomycorrhizal fungi. Cenococcum geophilum dominated, followed by *Lactarius pubescens, Inocybe maculata* and *Boletus luridus. Thelephora/Tomentella* was the dominant genus, followed by *Sebacina, Inocybe, Russula/Lactarius*, *Cortinarius* and *Hebeloma/Alnicola*. The area-based rarefaction curve and species richness estimates did not level off, indicating still too small sample size. The abandoned and managed wooded meadow plots shared only 18.6% of species, while O-horizon and A-horizon shared 53.5% of species. Only species richness per root fragment was higher in the abandoned wooded meadow. Species richness per plot, per sample and per fragment, as well as plot-based Jackknife2 richness estimate and Shannon-Wiener diversity index were higher in O-horizon than A-horizon. Detrended correspondence analysis revealed strong management effect, but negligible horizon and geographical effects on the fungal community composition. The frequency of Boletus spp., hypogeous fruiting fungi and melanized fungi was higher and the frequency of Sebacinaceae spp. was lower in managed wooded meadow plots. Differences in fungal communities were not explained by soil pH, P, Ca, Mg, K and total organic concentrations, suggesting that additional complex environmental variables are responsible for differentiation of the ectomycorrhizal fungal community. Tagamõisa wooded meadow comprises distinct mycoflora. More studies are needed to reveal if other wooded meadows support similar fungal communities.

PS5-508-0197

Marine-derived Fungi Isolated from Corals from Brazilian Coast for Bioprospection

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Marine-derived fungi represent a valuable source for the search of active metabolites of industrial interest, including compounds with potential application on pharmaceutical industry. They also have drawn attention for their capacity of degrading several pollutants. The fungal tolerance to higher concentrations of salt might be considered an advantage for bioremediation processes in marine environment. Therefore, corals collected from the sea town São Sebastião on the coast of São Paulo State, Brazil, identified as *Mussismilia hispida*, *Palythoa caribaeorum, Palythoa variabilis* and *Zoanthus solanderi*, were taken to the laboratory and were grinded and homogenized. Decimal serial dilutions up to 10-2 were performed with sterile water and aliquots were plated on marine agar and malt agar. Isolation of fungal colonies was conducted from the second day up to the fifteenth day incubation. The isolated fungi were separated into groups and were morphologically characterized. There were a total of 188 filamentous fungi represented by several species of *Penicillium*, including *P. citrinum, P. turbatum, P. purpurescens, P. montanense, P. corylophilum, P. mineoluteum, P. cyaneus* and *P. sublateridium*, species of *Aspergillus*, including *A. sydowii, A. versicolor* and *A. niger*, some representatives of *Mucor hiemalis, Fusarium spp., Trihcoderma spp., Peacilomyces sp*. and various unidentified dematiaceous and other hyaline fungi. These fungi are being tested regarding their potential to biodegrade toxic compounds, such as textile dye and polycyclic aromatic hydrocarbons. The selected fungi will be characterized by polyphasic approach, including molecular methods, in order to have a more accurate identification and will be deposited in the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI).

PS5-509-0198

Fungi Isolated from Sediment from Northern Region of Brazil and their Ability to Degrade Industrial Dyes

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The contamination generated by the textile industry has become a great problem confronted by the industrialized world of today. There are technologies for wastewater treatment, however they are very expensive and not very effective, becoming commercially less attractive. The biological treatment of textile effluents is an alternative more attractive due to its low cost, besides being more environmentally friendly. Fungi have been studied regarding their ability of bioremediating areas contaminated by several different toxic compounds, including textile dyes. Sediment samples from Manaus (Amazonas State) and Serra da Capivara (Piauí State), regions with a very rich biodiversity, were collected. Sediment samples were suspended in sterile water and added to enrichment media, after incubation culture samples were transferred to culture media containing different dyes at different concentrations. Following incubation, culture samples from flasks with visible decolorization were plated in agar media supplemented with dye subsequent to incubation the colonies surrounded by decolorized zones were isolated. The isolated fungi were tested in liquid media with 200 ppm of dye and degradation was measured spectrophotometrically. Several fungi were isolated and tested, among them *Fusarium sp.* and *Penicillium citrinum* were able to degrade efficiently the tested dye. The lignin-degrading fungus, *Lenitinula edodes*, was also evaluated and after 7 days incubation the dye degradation was complete. Toxicological assay, using the microcrustaceans *Daphnia pulex,* were also conducted and demonstrated the lower toxicity of the culture media with dye following treatment with these fungi. The methodology for selecting fungi from sediment samples was very efficient and the selected fungi are promising for bioremediating areas contaminated by dye effluents due to their efficiency for dye degradation and reduction of dye toxicity.

PS5-510-0209

Small mammal mycophagy within Phytophthora cinnamomi-affected heathland at Anglesea, Victoria, Australia.

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The tripartite relationship between plants, fungi and mycophagous mammals appears particularly vulnerable to disturbance. Understanding possible effects of disturbance factors may be crucial for the management of mycophagous small mammal species inhabiting such areas.

Phytophthora cinnamomi is a soil-borne pathogen that kills susceptible vegetation. In Australia, the effects of the pathogen on native vegetation have seen it listed as one of 11 key threatening processes to biodiversity under the EPBC Act, 1999. While the effects of the pathogen on vegetation, and to some extent fauna, have been widely studied, little is known of the effects on fungal communities.

A study assessing the effects of the plant pathogen P. cinnamomi on fauna and ecosystem function in heathland at Anglesea has included analysis of small mammal diets. In this study, scats of two small mammal species inhabiting the area were examined to survey fungal species diversity.

Scats of Rattus fuscipes (bush rat) and *Antechinus minimus* (swamp antechinus) were collected during live-trapping sessions conducted from 2002-2004. *R. fuscipes* is a well-known mycophagous species, however this is the first investigation into the occurrence of fungi in the diet of the predominantly insectivorous *A. minimus.*

Fungal species diversity was compared between seasons and over consecutive years. A total of 104 fungal spore types were recorded from scats of *R. fuscipes*, including the rare sequestrate species Richoniella macrospora. Thirty-seven spore types were recorded from scats of *A. minimus*, with four of these being unique to this species. The number of species recorded in this investigation is comparable to previous analyses in *P. cinnamomi* affected areas, thus inferring that Phytophthora-affected heathlands still appear to support a wide diversity of fungal species. This study emphasises that analysis of scats of ground-dwelling insectivores such as *A. minimus* can be a valuable contribution to studies of fungal diversity of a given area.

PS5-511-0222

Fungi of Azores: Corticiaceae s. l.

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With 480 km in length, the Azores archipelago is formed by nine main islands, with different dimensions, topography and altitudes, running in a NW-SE direction and rising from the submarine Mid-Atlantic Ridge. They are truly volcanic, of terciary origin, without connection to any major land masses. Its geographic position, in the middle of the Atlantic, 1250 km away from the nearest continent, allowed the flora and fauna that colonized these islands to evolve isolatedly, originating species and subspecies different from the continental ones. Since its discovery and consequent colonization by European settlers during the 15th century, the wildlife was severely affected by human activity, specially the flora, with the naturalisation and spread of many exotic species that grow exuberantly, benefiting from the mild and humid climate of the islands.
Very little is known about the mycology of the Azores, the earliest references are found in Drouet (Mém. Soc. Acad. Dép. Aube 3: 81-233. 1866), that listed 41 taxa of lichens and 2 ascomycetes. In 1977, Dennis et al. (Kew Bull. 32: 85-136), published a revision of the main contributions, with the addition of new registers to the Azorian mycological catalogue, including the 29 species of corticia until now referred for the archipelago.
800 samples of fungi were collected during a 9 day mycological foray at the end of the winter of 2005, in 3 Azorian islands of the central group, Faial, Pico and Terceira, in 23 localities situated at different altitudes. Part of the material was studied following classical methods and is kept in MA and LISU.
Until now, 100 species of corticia have been identified, 71 being new to the Azores. Most are cosmopolitan, but some are particularly interesting as *Dentocorticium sasae* (Boidin, Cand.& Gilles) Boidin, Lanquet. & Duhem, known only from France, Resinicium friabile Hjortstam & Melo, a South American species described from Brazil, *Tubulicium vermiferum* ssp. *raphidosporum* Boidin & Gilles, found in central Africa and Sri Lanka (Asia), *Tubulicium vermiculare* (Wakef.) Boidin & Gilles, known from New Zealand and Reunion Island (Africa), Conohypha terricola (Burt) Jülich and *Tubulicrinis sceptriferus* (H.S. Jacks. & Weresub) Donk, North American species in Europe registered only in Germany (Bavaria) and Vararia hauerslevii Boidin, very rare, with distribution restricted to northern Europe.
Considering the recent origin of the islands, the winter season and the short period of time of the foray, the number of identified species is relatively high, including fungi from different geographic areas. The main part of the material has not been studied yet, so it is expected that in the near future new species will be described, as happens with flora and fauna.

PS5-512-0227

Study on diversity of Aspergillus and Penicillium isolated From the mangrove forests of Vietnam and their potential application

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Aspergillus and *Penicilium* are cosmopolitan filamentous fungi. They play a very important part in Ecology and Industry. In Vietnam, so far several studies on Aspergillus and Penicllium of terrestrial land have been reported. However, the presence of these fungi in mangrove forests has not been studied. This work has been done in order to reveal the diversity of these two groups in mangrove forests and explore the gene pool from those fungi for practical use as well. *Aspergillus* species were identified by methods of Klich M. A., 2002 and Raper & Fennell, 1965; *Penicillium* species were identified by Pitt, J. I., 2001 and Rapper and Thom, 1949. Molecular sequencing of D1, D2 region methods were also applied for identification of several species. Hydrolytic enzyme and antibiotic activities were detected by using agar Block diffusion methods. From several mangrove forests in Vietnam, 75 strains of *Aspergillus* and 25 strains of *Penicillium* were isolated. The identification of those *Aspergillus* was done by conventional method and revealed that they belong to 25 species of 13 common *Aspergillus* species groups (*A. niger, A. flavus; A. ornatus; A. ochraceus; A.versicolor; A. ustus ; A. candidus; A. fumigatus; A.cremeus; A. sparsus ; A. terreus and A.wentii. Penicilium* species were identified to belong to 12 species of common *Penicillium (P. spinulosom Thom, P. islandicum Sopp, P. echinulatum Fassat, P. aurantiogriseum Dierckx, P. viridicatum Westling, P. crustosum Thom, P. expansum Link, P. chrysogenum* Thom*, P. corylophulum* Dierckx*, P. janthinellum* Biourge*, P. oxalicum* Curie and Thom, P. spinulosom Thom).
Interestingly, many unusual phenotypes of those studied species have been observed in comparison with the description of corresponding species in the references (in Raper & Fennell, 1965; Klich M. A., 2002, Rapper and Thom 1948, Pitt J. I. 2001). There were at least 25 strains of Aspergillus and Penicillium having a lot of mutations on conidia bearing structures.
Many mangrove *Penicillium* and *Aspergillus* species showed high capacity of hydrolytic enzyme and antibiotic productions. Notable one strain which was identified as *A. niger var .awamori* GM 57 could produce high amount of acidic xylanase and cellulase on Corn cob (xylanase of 5745I/g and cellulose of 84IU/g) showing potential application of this strain for industrial production.

PS5-513-0236

A United Database of fungal fpecimens in Japan

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Due to regional broadening in the South and North, Japan embraces a wide diversity of organisms. Fungi are no exception. In spite of a relatively long history of mycobiota inventories in Japan begun in early 1900s, much information remains to be added to the fungal inventory in Japan. Inventories require the mass accumulation of information, and advances in information technology and database software have contributed greatly to progress in this regard. GBIF (Global Biodiversity Information Facility), an international project, has provided a good opportunity to integrate information of specimens kept in the major mycological herbaria in Japan since 2002. The authors here present the outlines of the 4 years program to generate a united database of fungal specimens in some herbaria in Japan.
The following fungal herbaria and a culture collection participated and provided their data to the program: Kanagawa Prefectural Museum of Natural History (KPM), The Kyoto University Museum, Forest and Forest Products Research Institute (TFM), Tottori Mycological Institute (TMI), The National Science Museum (TNS), Plant Pathology Herbarium, Tsukuba University (TSH), Yamaguchi University (YAM), and Japan Collection of Microorganisms, RIKEN BioResource Center (JCM).
Currently some 32,000 records of fungal specimens have been cumulated. 78% of the specimens are basidiomycetes, followed by ascomycetes (13%), anamorphic fungi (6.3%), and others. The database can be accessed through the TNS homepage (http://svrsh2.kahaku.go.jp/fungal/). The record includes more than 6,000 species distributed among 1,400 genera. The majority are basidiomycetes (about 4,600 species) and ascomycetes (about 1,300 species). In addition, records of selected specimens with culture are linked to culture collection (JCM) database. The top 10 genera in the number of the specimens are: Puccinia, Ustilago, Amanita, Coleosporium, Hypoxylon, Russula, Coriolus (*Trametes), Uromyces, Melampsora,* and *Lactarius* in the order of number of specimens.
Analysing taxa diversity, basidiomycetes exceeds ascomycetes. However, the number of known ascomycetes is about twice of that of basidiomycetes based on literature data in Japan. The composition of the specimens in the herbarium does not reflect Japan's naturally occurring biodiversity, and increasing collections diversity is required. By analysing the data, localities rarely visited and taxonomic groups poorly represented became clear. Strategic collection to cover the locality for collection will be possible. Herbaria with historical specimens in the Northern part of Japan have not yet been incorporated, and further collaboration is desired.

PS5-514-0242

Some noteworthy species of *Tylopilus* (Boletaceae) from northern Queensland, Australia

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Tylopilus is a large, possibly polyphyletic genus of boletes typically distinguished by having pinkish-brown to vinaceous spore deposits. As part of a broad-scale (geographically and taxonomically) study of Tylopilus, we conducted a preliminary field study of the genus in early 2006 in northern Queensland (NQ), Australia, an area with expected high species richness that has been the subject of little research in terms of bolete diversity. Here, we present a study of three species that stand out in terms of their geographical distributions or morphological characters.
Tylopilus ballouii, originally described from the eastern U.S.A., appears to be a widely distributed species documented from both temperate and tropical regions. We encountered two distinct morphotypes in sclerophyll forests and rainforests containing ectomycorrhizal genera *Acacia, Eucalyptus*, and *Allocasuarina*: one having large, favoloid pores, the other having more typical small, round pores; we compare both morphotypes with North and Central American material using morphological and molecular data, and present a preliminary biogeographic hypothesis regarding the history of this species.
Xanthoconium separans is a distinctive species having a bright yellow brown spore deposit and turning bright turquoise in the pileus and stipe when dilute hydroxides are applied. In NQ, we encountered a taxon macromorphologically indistinguishable from *X. separans* with the exception of possessing a *Tylopilus*-like, pinkish-brown mature hymenophore. Here, we present a detailed comparison between this taxon (*Tylopilus separans*, nom. prov.) and X. *separans*, providing a commentary on the utility of spore deposit color as a diagnostic character at the generic level in the Boletaceae.
Tylopilus queenslandianus is a species morphologically similar to *T. chromapes*. We collected new material of T. *queenslandianus* from the type locality, and compare this taxon to T. *chromapes* and the morphologically similar Central American T. cartagoensis using morphological and molecular data.

PS5-515-0248

Wood-Decay fungi in CWD in logged and unlogged wet sclerophyll forests in Southern Tasmania

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Coarse woody debris (CWD) is regarded as a critical habitat for biodiversity in forest ecosystems. Communities of wood-decay fungi may constitute the prime agents of wood decay and hence the drivers of ecological succession within CWD, but are poorly understood in Australia, where the biodiversity associated with CWD has not been as well studied as in some northern temperate regions.
This study took place in the cool temperate wet eucalypt forests in southern Tasmania. It examined the wood-decay fungi in large (>85cm) and small (30-60cm) diameter Eucalyptus obliqua logs in mature, unlogged forests and in forests that were regenerating following clearfelling 20-30 years previously.
Selected logs, all of an intermediate decay stage, were cross-cut at three standard points along their length. Where decayed wood was found, samples were taken and incubated on specialised media to isolate the associated wood-decay fungi. The identity of these isolates was then determined using a combination of morphological characters and sequencing data based on the Internal Transcribed Spacer Region of rDNA. Relationships among fungal community composition, forest type and log size were examined.
A total of 135 species of wood-decay fungi were isolated from the 36 logs examined. Several of these fungi are thought to be new species. Significant differences in fungal community composition were found between logs in mature versus regenerating forests. Further differences were also found between logs of different sizes.
The findings from this research will assist in the development and deployment of strategies for the sustainable management of CWD and its dependent biodiversity in wet eucalypt forests in Tasmania.

PS5-516-0250

Decayed wood, wood-inhabiting fungi and saproxylic beetles in living *eucalyptus obliqua* trees in Southern Tasmania

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Southern Tasmanian wet sclerophyll forests dominated by *Eucalyptus obliqua* are managed on a notional rotation length of 80 to 100 years. Over time, this may reduce the proportion of old living trees within the production forest landscape. This project explored the extent to which such a change might impact on wood-inhabiting fungi and saproxylic (dead-wood dependent) beetles, as important but often overlooked components of forest biodiversity.
Six living E. obliqua trees in each of three age-classes (69, 105 and >150 years old) were felled and cut into sections. The decay profile of each section was recorded, and fungal cultures derived from samples of each visible decay type. A parallel study sampled saproxylic beetles from the same sections, using emergence traps and hand searching.
Ninety-one species of wood-inhabiting fungi were isolated from the 18 trees. The fungal assemblages in trees in the oldest age-class were very different from those found in those in the younger two age-classes; more than half of all species were only found in these older trees. Trees in the oldest age-class also contained greater volumes and proportions of decayed wood habitat. One hundred and sixty saproxylic beetle species were collected. The number of individuals and species increased with tree age, with significantly higher numbers of species found in trees in the oldest age-class. Assemblage composition of saproxylic beetles also changed with tree age class.
This research suggests there is a need for forest managers to consider instigating measures that allow for some trees in the production forest landscape to live long enough to develop decayed wood habitat and hence to provide important habitat for sustaining an important component of forest biodiversity.

PS5-517-0252

Fungal Diversity On Leaf Litter

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The present investigations deals with mycoflora colonizing the leaf litter of *Citrus aurantium L. Achras sapota l. Swetenia mahagoni L*. and *Acacia melanoxylon* R.Br. The fungi were grouped as 'Dominant','Common' 'Frequent', 'Occasional', and 'Rare', depending on their percentage frequency only a few fungi appeared to be 'Dominant' on each plant species and nearly half the number of species occurred sporadically. Each plant had its own characteristic 'Dominant' mycoflora, which include mostly host specific forms. Many species were found to be common to all the four plant species, but the frequency and percentage occurrence of these species was different on four litter types.

PS5-518-0265

On The Diversity of *Isaria* species from Thailand

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All species of the resurrected genus *Isaria* previously belonged to Section Isarioidea of *Paecilomyces* sensu lato. Isaria is an entomogenous genus with colonies appearing in bright colors: white, yellow, orange or red. The conidiophores could be mono- or synnematous, consisting of verticillate branches, bearing dense whorl of phialides. On the insect host the phialides, though flask-shaped, do not always possess the long neck they have in culture. Conidia are produced in long divergent chains, usually one-celled, smooth or ornamented and hyaline or pale pink in color. The perfect state is never seen in culture but associations with the Cordyceps teleomorph have been seen in nature. Of the ten species recently placed in the genus, seven have been collected in Thai forests. These are I*saria tenuipes, Isaria javanica, Isaria cicadae, Isaria fumosorosea, Isaria amoenerosea, Isaria farinosa*, and *Isaria ghanensis*. Four of these, *I. tenuipes, I. javanica, I. amoenerosea* and *I. farinosa* are most commonly encountered. Recent molecular studies based on ITS rDNA have shown, however, that strains identified as such are mixed with other species within the genus, suggesting of morphological phenotypic simplicity or species complexity.

PS5-519-0271

Studies On Litter Fungi: Fungal Colonization Of Ficus benjamina L. and Gliricidia maculata HBK

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The pattern of fungal colonization of leaves *Ficus benjamina* and *Gliricidia maculata* was investigated by examining three categories of leaves (G1, G2 and G3) representing progressive stages of decomposing litter. Greater number of species was found on G1 and G2 than on G3 litter. Many species were common to G1 and G2 but only 50% of these appeared on G3 litter. Although some species were found on all the three categories of litter their colonization efficiency was not the same. No species appeared afresh on G3 litter. That some of the colonizing the leaves from the phylloplane stage onwards continues to persist, and new one also appears at different stages of decomposition is evident from the data, which suggests the colonization, and persistence is a continuum.

PS5-520-0282

Identification of *Armillaria* (Basidiomycetes, Agaricales) species in forest biotops of Central Europe from soil substrate

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The genus *Armillaria* induces serious root rot disease of European forest stands. There are seven different species in Europe which differ in pathogenicity. The purpose of the study was to analyse the distribution of *Armillaria* from soil samples in different forest ecosystems and to identify the fungi at the specific level. The four study sites were chosen and five plots were established there. The study plots differed in its tree species composition and treatments of forest management. In this study, the DNA–based methods were used for detection of Armillaria in soil. The *Armillaria* specific primer pair (AR1, AR2) based on conserved sequences within the so-called ITS region (ribosomal DNA) was used for direct amplification of DNA from soil samples by nested PCR. The individual species were distinguished by RFLPs analysis with restriction endonuclease Hinf I. The results confirmed that different species coexist sympatrically in the same forest stand and the Armillaria species composition depends on type of forest ecosystem. The work was supported by Grant agency of the Czech Republic, project no. 526/05/0086, and by Ministry of Education, Youth and Sports, project no. MSM 6215648902.

PS5-521-0285

Mycocoenological characterization of evergreen oak forests in the Basque Country (North Spain) and its relation to biotic and abiotic factors.

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Macrofungi as heterotrophic organisms depend on green plants for survival, and it is recognized that plant communities determine the distribution and composition of macrofungal communities or mycocoenoses. In fact, plants conform the habitat and energy resource for fungi.
The Basque Country is located in the north of Spain and is just on the border between the Mediterranean and Eurosiberian region. The northern part of the territory belongs to the Eurosiberian Region and the south part to the Mediterranean. In less than 100 km climatic conditions change in the territory, more rainy and mild temperatures in the north and less rainy and more extreme temperatures towards the south.
Evergreen oak forest is the typical mediterranean plant community, but in the Basque Country this community can be found in the Eurosiberian region where the soil is badly developed and drains quickly. The evergreen oak forests change on the north-south gradient, *Lauro nobilis-Quercetum ilicis* association in the Eurosiberian region and *Spiraeo obovatae-Quercetum rotundifoliae* in the Mediterranean region. This last association is divided in two subassociations, subass. *quercetosum rotundifoliae* in the transition zone and subass. *arbutetosum unedonis* in the south part. The aim of this study was to analyse the macrofungal communities in evergreen oak forests and also to see how very similar plant communites influence the mycocoenoses.
Four permanent plots of 400 m2 (20 x 20 m) were delimitated in 6 different evergreen oak forests. Seven plots in the north, eight plots in the middle or transition zone and eight plots in the south. Each plot was visited every week the first year and every fortnight the following four years. During the visits the macrofungal species were recordered and each carpophore counted. Phytosociological releves were done in each plot, and different structural and edaphical factors were measured.
Muyltivariate analyses (MDS, CLUSTER, ANOSIM, RELATE, LINTREE) were conducted to see differences in mycocoenoses of different plant communities and relate mycocoenosis to phytocoenosis and environmental factors (Primer-E , clark)
A total of 386 species were found after four years of sampling. Ordenation and classification analyses show a gradient in the mycocoenosis in a north south transet which is correlated with the distribution of different plant associations. These results show that fungal community is closely related to plant community and different plant association or subassociation affect mycocoenoses. Nevertheless, the fact that the ectomycorrhizal community is also correlated with the distribution of plant community, and taking into account that ectomycorrhizal plants are the same in all the plots, makes us think that other factors apart from plants are responsible for the differences between zones. Indeed, these factors could also be responsible for the distribution of plant communities. Canopy, slope, pH and sand concentration have been found to explain the variability in the distribution of mycocoenoses in evergreen oak forest.

PS5-522-0301

Earthstars of the Hawaiian Islands: the five large *Geastrum* species.

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This study is part of an ongoing study of the gasteromycetes of the Hawaiian Islands. As far as the earthstars are concerned, eighteen species of *Geastrum* and the monotypic genus *Myriostoma* have been located, some within dry, leeward coastal and montane regions that only receive periodic rain and others in wet windward coastal and montane regions that are constantly moist. There has been a controversy over the identification of some of the larger *Geastrum* species in Hawaii, but with the collection of large number of specimens, including primordia, five distinct species with large endoperidia can be identified: *Geastrum fimbriatum, G. morganii, G. rufescens, G. triplex,* and a yet to be described species that is given the provisional name G. "*litchi*" for its close resemblance to the fruit of the litchee tree, Litchi chinensis. The distinctive features used to identify and separate these species, including growth habits, adhering debris, and peristome details will be described. A key to all the earthstars found thus far in the Hawaiian Islands and their preferred habitats will be included in this presentation.

PS5-523-0309

Impact of logging on wood decay fungi in fine woody debris in a New Zealand forest

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Despite fulfilling important roles in decomposition and nutrient cycling, there is a paucity of information on the diversity and distribution of wood-decay fungi in New Zealand's natural ecosystems. Podocarp-broadleaf forests of New Zealand's Urewera Ranges form a mosaic of selectively logged and undisturbed forests and present an opportunity to study fungal diversity and distribution across sites of different logging histories.
Methods: Four sites were selected to survey macrofungal fruiting bodies in fine woody debris. The sites were matched for altitude and forest type and included two which had been selectively logged about three decades ago, and two which had never been logged. Logged sites did not show any obvious signs of recent disturbance and did not differ significantly in the overall number of trees, but had fewer tree species and a denser canopy. Eight subplots per site were sampled in spring (October) and autumn (May). Sampling was restricted to macrofungal fruiting bodies on fine woody debris.
A total 428 fungal occurrences were recorded, which represented 136 taxa. Among these, 14 taxa were new records for New Zealand. A small number of taxa among ascomycetes and heterobasidiomycetes dominated the observed fungal communities. Other basidiomycetes were proportionally underrepresented among common taxa. A high percentage of taxa (57 %) were observed from a single specimen. Fungal communities at sites which had been selectively logged over 30 years ago had significantly fewer taxa and fewer fungal records compared to sites that had never been logged. A higher percentage of taxa appeared restricted to unlogged compared to logged sites. In addition, fungal communities at logged sites displayed a higher multivariate variability than those at unlogged sites.
It is hypothesised that the lower species richness at logged sites may be related to the lower diversity among substrata, although other influences such as a decrease in light cannot be excluded. Decreases in species richness and increases in variability have been interpreted as indicators of stress in communities of other ecosystems, such as marine and benthic environments. Further work is needed to confirm that these measures also represent indicators of stress in fungal communities.

PS5-524-0315

Factors shaping microfungal communities in litter in Australian wet tropics rainforest

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Saprobic microfungi of leaf litter represent an understudied but highly diverse group of fungi. The distribution of microfungi in this habitat has not been well characterised, particularly in tropical regions. This study explored the diversity and distribution of leaf litter microfungi in selected hosts in the wet tropics of Australia as a preliminary step to better understand factors involved in shaping microfungal communities of this region.
Over a two-year period, microfungi were observed in leaf litter of tree species belonging to four common plant families of the region, namely the Elaeocarpaceae, Lauraceae, Moraceae, and Proteaceae. Sampling was undertaken at two sites, which were matched approximately for rainforest type, altitude and rainfall. Fungi were studied using two approaches, isolation of cultures by particle filtration and direct observation of fruiting bodies following incubation in humid chambers.
Despite detecting a high variability between samples and a high percentage of singleton species, multivariate analyses showed clearly that microfungal communities differed significantly between plant families and also between seasons but not between sites. The greatest similarity was observed between microfungal communities on congeneric tree species, followed by those on different genera in the same plant family and finally by those on different plant families. Species richness differed significantly between some of the tree species and was negatively correlated with the concentration of total phenolics as determined for living leaves. Positive correlations were observed for species richness with leaf thickness and manganese concentration.
Host phylogeny was the most important factor seen to shape microfungal communities and, at the sample size used in this study, was significant at the level of host family. A degree of host preference among saprobic fungi detected in the present study supports previous observations by mycologists that some saprobic fungi can be host specific. However, host preference observed in the present study was not restricted only to apparently host specific fungi, but was also noted for cosmopolitan species reported previously from a wide range of substrata. One might hypothesise that physical and chemical characteristics of decaying leaves contribute to patterns indicating a host preference. The great variability between samples points to chance and other factors not measured in our study as important influences in shaping microfungal distributions. Further work is required within the framework developed in this study, to increase our knowledge on microfungal communities since understanding microfungal distributions and host preference will ultimately assist in refining global diversity estimates.

PS5-525-0325

Environmental Isolation of Entomophthorales from South Australia

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This report documents the novel isolation of *Conidiobolus* and *Basidiobolus* species from an environmental niche in South Australia. As a result of the incidental isolation of *Condiobolus coronatus* from local spring water, two high altitude bogs were investigated to determine the natural source of Entomophthorales.
Twenty samples of approximately 5-10 grams of soil and leaf litter were collected from the Adelaide Hills spring water property and Wilson's Bog in the Greater Mount Lofty Park. The isolation technique of Shipton and Zahari (J Med Vet Mycol 1987:25,323-327) was used. Briefly 5 g of sample was suspended in 5 ml sterile distilled water, vortexed and 1.5 ml dispensed onto sterile filter paper placed in the lid of a 9 cm Sabouraud dextrose agar plate. Plates were placed upside down with the agar surface the focus of discharged spores, incubated at 25 C in natural light.
Six samples from the spring water property yielded 4 isolations of *C. coronatus,* one *C. thromboides* and one *Basidiobolus ranarum*. While Wilson's Bog yielded one isolation of *C. coronatus* and one *B. ranarum*. Isolates were identified by typical colonial and microscopic morphology and confirmed by PCR and DNA sequencing of the internal transcribed spacer regions (ITS) and part of the small subunit (18S) r RNA. Sequencing was performed by the ICPMR laboratory, Westmead Hospital, Westmead, N.S.W.
Conidiobolus and *Basidiobolus* species have been isolated from soil, plant detritus and reptile dung. In Australia, animal infections have been reported from the Northern Territory, Queensland and northern New South Wales. Isolation of or infection acquired in South Australia by Entomophthorales has not been previously reported. In this study *Condiobolus* and *Basidiobolus* species were isolated from both sites, both high altitude bogs with permanent spring water and unique native flora. This suggests that the original isolation did not represent a contaminated site but more likely a restricted natural source. Lack of other reports or incidental isolations suggests that these fungi occupy a special niche in S.A. Further sampling of other habitats is suggested.

PS5-526-0336

Assessment of river environment using *Pythium* species

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Although animals and plants have been widely used as an indicator in an environment assessment, studies on the use of microorganisms are still limited. Since microorganisms are able to respond fast to environmental changes, it may be a good indicator for an environment assessment. *Pythium* is a worldwide-distributed genus, consists of saprophytes, animal and plant pathogens, and mycoparasites. Therefore, we investigated the feasibility of *Pythium* species to be used as an indicator for an assessment of river environment.
Soil samples were collected from Japanese pampas grass colonies in river basins of Nagara, Kiso and Chikugo Rivers in Japan. Soil dilution method was applied to isolate *Pythium* species on *Pythium* selective medium.
Twenty species and five groups were isolated from the three rivers. Group HS isolates were subsequently classified into six strains, HS1 to HS6, in phylogenetic analysis of the internal transcribed spacer region of rDNA. Most of the species except for the HS2 strain and *P. irregulare* did not show any trend in their distribution. The inoculum density for the HS2 strain was higher in upstream, while *P. irregulare* was higher in downstream in all of the three rivers. Temperature did not influence the distribution of the HS2 strain and *P. irregulare*, since they showed similar optimum, maximum and minimum mycelial growth temperatures. Inoculum densities of the HS2 strain and *P. irregulare* did not correlate to soil texture and pH. The density of the HS2 strain in soil was positively correlated to the area of forest and the degree of naturalness. The density of *P. irregulare* was positively correlated to the area of farmland and negatively to the degree of naturalness. The results suggest that in an environmental impact assessment of river, the HS2 strain can be used for an evaluation of degree of naturalness, while *P. irregulare* for an evaluation of the impact level of agricultural activity.

PS5-527-0341

Pythium species in cool-temperate forest soil

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Pythium is a worldwide-distributed genus, contains more than 120 species and consists of saprophytes, animal and plant pathogens, and mycoparasites. In this study, we investigated *Pythium* species inhabiting in cool-temperate forest soils in Japan.
Materials and Methods Soil samples were collected from seven vegetations in Takayama, Japan, in winter and summer. Soil dilution method was applied to isolate *Pythium* species, using *Pythium* selective medium. Identification was performed based on morphological characteristics, phylogenetic and RFLP analyses of the internal transcribed spacer (ITS) region of rDNA.
P. *spinosum, P. sylvaticum*, a new species, and *Pythium* group HS forming hyphal swellings with no sexual organ were isolated. Group HS isolates were classified into four strains, HS1, HS2, HS3, and HS7, in RFLP analysis of the ITS region according to Tanahashi et al. (2004). The HS2 strain was predominant in all vegetations, even though the strain has a slower growth than *P. spinosum* and *P. sylvaticum*. The predominance of the HS2 strain might reflect its strong saprophytic and competitive ability in soil.
Inoculum density of *Pythium* species was highest in mixed forest soil, regardless of seasons, followed by evergreen coniferous forest soil. On the other hand, the density was low in deciduous coniferous and deciduous broad-leaved forest soils, also regardless of seasons. It seems that litter supplement in any season is sufficient for the enhancement of saprophytic activity in mixed and evergreen coniferous forest soils. Especially, the HS2 strain that forms no sexual organ, which is a resistant survival structure, has an advantage to grow continuously in organic matters in such forest soils.
Inoculum density was higher in upper layer of soil than in lower layer, confirming that *Pythium* species play a role as a decomposer of soil sugars at the earlier stage of organic matter decomposition.

PS5-528-0345

Diversity and distribution of polypores in Northern Thailand

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Hjortstam and Ryvaraden (1982) reported about 100 species of polypores from Northern Thailand based on the collections during a short expedition. But there are no other intensive reports from this region and polypore flora is far from completed in Northern Thailand.
We examined polypore specimens collected mainly in and around Chiang Mai Province. Among them, a number of species are hitherto unreported from Thailand. Distribution pattern of the collected species was discussed.
Species collected in lowland forests (alt 0-800 m) are tropical species that are widely distributed in tropical areas of Asia. Some species as follows are also common in warm temperate areas of Asia: *Microporus affinis, Perenniporia ochroleuca*, and *Phellinus gilvus*. Among the collected species in hill forests (alt 800-1500 m), many species are tropical elements including those also distributed in temperate areas. Some species as follows are temperate species that are unknown from tropical areas: *Cyclomyces fusca, Gloeophyllum subferrugineum*, and *Pyrrhoderma adamantinum*. In highland forests (1500- m), tropical species such as *Lenzites acutus* and *Nigrofomes melanopus* are seen together with temperate species such as *Antrodiella zonata* and *Inonotus flavidus* that are never found in subtropical and tropical areas. Additionally, *Ceriporia subreticulata* is hitherto known only from highland forests in Thailand, and some of other undescribed species found there are also expected to be endemic in highlands of the Southeast Asia.
In low latitude areas, polypore flora is very different between lowlands and highlands. There are some well-reserved mountain forests in Northern Thailand. Polypore diversity should be high in this area with tropical elements, temperate elements and endemic species in highland forests. Several undescribed species are also expected both in lowland and highland forests.

PS5-529-0349

Plant Diseases Herbarium in Thailand

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Thailand Plant Diseases Herbarium, Department of Agriculture was established in 2003 by the collaborative project of Thai-Australia Government Sector Linkages Program (TGSLP) and Plant Pathology Research Group, Department of Agriculture, Thailand. There are over 1,500 specimens of plant diseases in herbarium collection. About 900 Specimens collected during recent surveys of plant diseases in Thailand, and 600 specimens were collected before 2003. Most of collection specimens were plant disease infected by fungi, such as leaf spot, rust, powdery mildew, downy mildew, sooty mould, etc. The main objectives of the Plant Diseases Herbarium; to be the center for plant diseases specimens in Thailand, to be a source of informations of plant diseases in Thailand and to be the basic supporting data for plant pest lists and pest risk analysis.

PS5-530-0350

Fungal diversity of decomposing fruits and seeds in tropical forests in Thailand

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Three fruit types, namely *Dipterocarpus turbinatus, Delonix regia*, and *Chlorospondias axillaris* were selected to study decomposition rates and fungal colonization by using a litterbag method at 2 sites in an evergreen forest at Khao Yai National Park, Thailand. One hundred and one fungi were identified with the majority litter fungi that are usually found on decaying plants. Common species to the three fruits were *Dictyochaeta* sp., *Thozetella nivea* and *Dinemasporium lanatum*. However, fungal communities differed on the selected fruits. *D. turbinatus* supported 37 species (dominant species: *Cryptophialoidea secunda, Dictyochaeta* sp. and *Thozetella nivea*); D. *regia* 61 taxa (*Dictyochaeta* sp and *Phaeoisaria clematidis*) with only 25 species on *C. axilliaris (Dinemasporium lanatum* and *Thozetella nivea)*. The fungal communities on decaying D. turbinatus fruit and C. axillaris seeds at the 2 sites were similar but differed on D. regia fruit. Furthermore fungi colonizing the fruits and seeds were classified into regular inhabitants, early and late colonizers, while some species were inconsistent in their occurrence. Fifteen fungi are new records for Thailand with *Cirrenalia nigrospora* a new species on *D. regia* fruit. The decomposition study showed that *D. turbinatus* fruits decayed completely within 1 year of exposure (k=4.6), while weight loss of *D. regia* fruits was 95-97% in 12 months (k=2.98-3.38). However, only 10-11% weight loss was recorded for *C. axillaris* seeds over 12 months (k=0.10-0.11). Dry weight losses positively correlated with monthly rainfall and relative humidity, while fruits and seeds with a higher C:N ratio decomposed slower than those with lower ratios.

PS5-531-0392

Aquatic hyphomycetes – diversity and ecobiochemical response in polluted habitats

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Aquatic hyphomycetes (AQH) (mitosporic fungi) generally dominate leaf decomposition in unpolluted ecosystems. An amazing diversity of AQHs was found in highly heavy metal polluted surface and groundwater of a former copper mining district of Central Germany [rev. Krauss et al. 2005]. AQH strains isolated from different polluted waters emphasize that adaptation to heavy metal exposure is quite specific, and tolerance to one metal does not confer a general resistance.
Together with certain morphological characteristics, biochemical and physiological divergences in heavy metal biosorption and their intracellular accumulation, cellular oxidative balance, and fungal thiol metabolism (induction of phytochelatin 2 and a novel small metallothionein) suggest that some strains may represent ecotypes, where distinct genetic and physiological adaptions to differentially contaminated habitats have been evolved.
Summarizing, aquatic fungi can play an important ecological role in alleviating environmental pollution in different ways. Thus, they also represent promising candidates for natural attenuation and bioremediation purposes.
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PS5-532-0412

Conservation of non-lichen forming microfungi

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The conservation community is currently able to provide comprehensive global information on the conservation status of just three classes of organisms: mammals, birds and amphibians. Information on trends in extinction risk are only available for two: birds and amphibians. In total, these represent only about 1% of the world's described species (and a much smaller proportion of the probable total biodiversity, which would include huge numbers of undescribed fungi and invertebrate animals). At present, little can be said about the status or extinction risk trends of the other 99% of described species. The IUCN's Sampled Red List Index project has been developed to address this major gap by aiming to provide information on conservation status and extinction risk trends which is more representative of these other species groups. To do this, a small number of major groups has been identified as targets.
Three of those groups constitute the fungal component: the non-lichen-forming microfungi, the lichen-forming fungi, and the basidiomycetes (ie macrofungi or mushrooms & toadstools). The present abstract deals with the first of these, ie the non-lichen forming microfungi. In collaboration with the IUCN, three independent prototype specialist conservation groups are being set up for:
• non-lichen forming ascomycetes and anamorphic fungi;
• rusts & smuts;
• chromistans, chytrids, myxomycetes and zygomycetes.
Each group will focus attention on conservation of its fungi, and will prepare information on the status of a randomly selected sample. Although the groupings are somewhat artificial, they represent a first step towards identifying the conservation status and needs of a group of organisms in theory protected under the Rio Convention on Biological Diversity, but in reality up to now almost totally sidelined by the conservation movement. For more information, please visit the website: http://www.cybertruffle.org.uk/iucn_red_list/index.htm.

PS5-533-0421

Macromycetes of the Prosisko Natural Reserve

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The Prosisko National Reserve is situated in the middle of the Slovak Republic nearby the town Zvolen, within an area of Zvolenska Slatina village. The main reason for the declaration of that relatively small area for a reserve in 1998 was the protection of forest association with concentrated presence of the preglacial herbaceous species Waldsteinia ternata ssp magicii. The highest degree of protection is related to this area and any encroachment to the biotic or abiotic part of ecosystem is permitted. Fungi – the natural and inevitable part of nature, are very sensitive to changes of ecological conditions. There is an opportunity to observe the changes of a fungal species spectrum in connection to both various growing conditions and changing forest community.
The forest stand under study (surface area 20,54 ha, average age 100 years) is created firstly by oak *Quercus petraea* (85%), hornbeam *Carpinus betulus* (15%) and sporadically by maple, beech, lime, cherry, rowan, hazel, alder and hawthorn.
The research was concentrated to four 50 x 50 metres monitoring plots (MP1 – MP4) situated in the characteristic places of the forest stand.
There were found 154 species of macrofungi including 34 species of ectomycorrhizal, 44 species of terrestrial saprophytic, 66 species of wood saprophytic and 10 species of sapro-parasitic ones. The initial results indicate by means of the mycorrhizal percentage (Gulden et al. 1992) and through the ratio of mycorrhizal and wood-destroying fungi considerable stage of disturbation of the ectotrophic stability of a forest stand (Soukup 1997).
The presence of macromycetes will be compared to progression of the health condition of trees during next years (Pavlik 1999, 2002).
Acknowledgement: The authors thank the Slovak Grant Agency for Science VEGA for a financial support of the research (grant No. 1/1328/04)
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PS5-534-0423

Worldwide movement of horse chestnut (*Aesculus hippocastanum* L.) anamorphic leaf pathogens: monitoring in Ukraine

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About 34 anamorphic fungi are associated with *Aesculus hippocastanum* L. These fungi constitute the biggest single group causing diseases of this tree. Spread of horse chestnut pests such as the leaf-miner in Europe over recent years has stimulated more precise assessments of fungal leaf pathogens. Four types of fungal diseases are known: leaf spot and blotch, wilt, scorch, and mould. The occurrence of anamorphic fungi on horse chestnut leaves was monitored in Ukraine from 2003 to 2005, and 10 anamorphic fungi were found in different regions. These were *Ascochyta grandimaculans* Kabát & Bubák, *Botrytis cinerea* Pers. (Botryotinia fuckeliana (de Bary) *Whetzel* teleomorph), *Passalora aesculina* (Ellis & Kellerm.) U. Braun & Crous, *Cladosporium herbarum* (Pers.) Link, *Geotrichum candidum* Link (Galactomyces geotrichum (E.E. Butler & L.G. Petersen) Redhead & Malloch teleomorph), Phyllosticta paviaecola Brun., *Guignardia aesculi* (Peck) V.B. Stewart teleomorph with *Phyllosticta sphaeropsoidea* Ellis & Everh. and *Leptodothiorella aesculicola* (Sacc.) Sivan. synanamorphs, *Septoria glabrae* Ellis & Everh., *S. hippocastani* Berk. & Broome., *Torula herbarum* (Pers.) Link . Leaf blotch of *Guignardia aesculi* and leaf spot of *Septoria hippocastani* were the main pathogens and can almost totally defoliate trees by through premature leaf fall in the northwest, centre and southeast of Ukraine. *Guignardia aesculi* has a wide European-North American distribution, whereas *S. hippocastani* has a Eurasian-North American distribution. Sensitivity to air pollution, salt injury to roots, herbicides and dry winds makes horse chestnut susceptible to development and spread of new fungi under climate change conditions. Thus *Ascochyta grandimaculans* has been observed spreading from the Czech Republic and Germany to the Carpathians, and another route through Romania was detected in 2005. Cercospora aesculina, collected in northeast Ukraine, has moved in from North America having long been predicted to arrive in Europe. Some species or anamorphic stages of *Colletotrichum, Cylindrosporium, Phyllosticta, Verticillium* remain more characteristic for North America.

PS5-535-0424

The Altai mountains as an area of missing and rare anamorphic fungi

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New information from unexplored areas of the world can change views on the distribution and migration of fungal species. One such area, in Temperate Asia, is Altai with its unique highlands, different climate and high level of biodiversity. About 170 species of anamorphic fungi have been recorded from the Altai Mountains since collecting started in the 1920s (Murashkinsky, 1924, 1926; Murashkinsky, Ziling, 1928; Melnik, 1988, 1989, 1997; Shkarupa, 1989, 1992). A new survey of leaf-inhabiting anamorphic fungi was carried out during 2000 in six main vegetation types of North Altai: taiga, mixed forests, tundra, bogs and marshes, steppes, and meadows. Some fungi found had not been collected since K.E. Murashkinsky's early investigations. Disjunctions in world distribution of *Pseudocercospora salicina, Ramularia primulae, R. ulmariae, Seifertia azaleae, Ascochyta tennerima, A. volubilis, Coniella australiensis, Hainesia lythri, Septoria linneae, S. nepetae, S. scabiosicola* and *S. urticae* were filled. In the Altai mixed forests, *Pseudocercospora saniculae-europaeae* and *Quasiphloeospora saximontanensis*, rare species known from other continents and sometimes interpreted as "missed", were found. Tundra in the highlands and taiga of Abies sibirica were sites for *Ascochyta volubilis*, *Phoma phlomidis*, *Septogloeum veratri, Septoria fulvescens* and *S. linneae*. *Myxothyrium leptideum*, *Septoria stellariae, Sporonema punctiforme* and other plant pathogens were observed in mountain taiga sphagnum bogs. Hyphomycete numbers were highest in steppe ecosystems, where several "missed" species were found. These new records throw new light on fungal migration. The records of *Phoma cannabis*, *Septoria cannabis, S. urticae*, weed pathogens common in Altai, help to reconstruct the migration routes followed during their global expansion. 32 species of leaf-inhabiting anamorphic fungi were observed for the first time in Asia, Russian Asia and Altai Mountains. New species of *Cordana, Robillarda* and *Septoriella* were found and are being described.

PS5-536-0426

Micromycetes inhabiting soda solonchaks and halophytic plants in Central Asia

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The saline alkaline soils represent unique extreme environments. They contain high to extremely high concentrations of soluble salts such as sodium carbonate/bicarbonate, sodium chloride, sodium sulfate and offer high pH values. In contrast to (halo)alkaliphilic microorganisms of soda lakes, mycological studies of soda soils are very scarce. Almost nothing is known about the micromycetes inhabiting soda soils.

The research was focused on saline alkaline soils surrounding lakes in Central Asia - Kulunda Steppe of Altai, Baikal lake region, Aral lake region and Gobi desert in Mongolia, ç 7 - 10,3. Total 81 samples of saline soda soils and 19 samples of halophytic plants *Anabasis* salsa, *Atriplex verrucifera, Halocnemum strobilaceum, Salicornia europaea* and *Suaeda spp.* were examined. Small pieces of soil (10 mg) as well as parts of plants were placed on Petri dishes with nutritional alkaline agar (AA) with soda buffer. Antibiotics rifampicin (2 mg/l) was used. By means of soda buffer pH was maintained at 10-10.,5.

About 200 isolates corresponding to 43 fungal species were isolated on AA from samples. Fungal community was not very rich and included predominantly hyaline and dark colored Mycelia sterilia, Acremonium spp., Verticillium spp., *Tilachlidium* spp., *Heleococcum alkalinum, Scopulariopsis* spp., *Lecanicillium* spp. and some others dominated there. The genus Acremonium was represented by 14 species - A. alkalophilum, A. bactrocephalum, A. biseptum, A. charticola, A. *kiliense,* A. *psammosporum*, A. *pseudozeylanicum, A. roseogriseum, A. roseolum, A. rutilum, A. salmoneum,* A. *strictum, Acremonium anam. Nectria sp., Acremonium sp*.. The predominant part of haloalkalitolerant fungal community includes anamorphic Hypocreales. One of the dominants was unusual and it was described as a new species – *Heleococcum alkalinum Bilanenko et Ivanova* (Ascomycetes, Hypocreales, Bionectriaceae) with *Acremonium* sect. *Nectrioidea* anamorph.

It may be supposed that some species are tend to vegetal substrates, while others to shrimp residues. So, we may conclude that the haloalkalitolerant micromycetes form an important part of the hydrolytic community even under the extreme pH values and salts concentrations. This vivid demonstration of biodiversity of fungi is really inspiring for further researches aimed at discovering new species with uncommon physiological potentialities. They could be analyzed as a potential producers of new metabolites and represent a unique model for future investigations of halo-alkaliadaptation mechanisms.

PS5-538-0467

Diversity and distribution of foliar fungal endophytes in New Zealand podocarps.

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The diversity and distribution of fungal endophytes in four conifers in the family Podocarpaceae (Dacrydium cupressinum, *Prumnopitys ferruginea*, *Dacrycarpus dacrydioides* and *Podocarpus totara*) and an angiosperm (*Kunzea ericoides, Myrtaceae*) occurring close to the sampled trees was studied. The effects of host species, locality and season on endophyte assemblages were investigated. Two trees of each host were sampled from each of the five sites in both summer and winter. The five sampling sites were distributed across two geographical regions in the North Island of New Zealand; four in the Waitakere Ranges near Auckland and one near the Te Urewera National Park, west of Gisborne. Endophytes were isolated from 50 leaves or a total of 200 leaf pieces per tree. A total of 4922 endophyte strains representing 479 morphotypes were recovered in winter and 4045 endophyte strains representing 495 morphotypes were isolated in summer. Endophyte assemblages were strongly shaped by host species, and to a lesser extent by region and season. There was no evidence for family-level specialisation across the Podocarpaceae where the mycobiota of each host species was characterised by a small number of dominant fungi. These dominant species were usually often observed on some of the other hosts although at low frequencies. The less host restricted endophyte species, found on several of the podocarps, occurred at similar levels on Kunzea. Although a few endophytes were dominant in summer and others in winter, overall difference in their assemblages between seasons was small. We conclude that the most important factor in shaping endophyte assemblages in members of New Zealand Podocarpaceae is the host species, followed by geographical separation and by seasonal effects.

PS5-539-0475

Seasonality of hypogeous fungi availability – implications of global climate-change

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Despite their importance to ecosystem health and function, and their contribution to global biodiversity, fungal organisms are generally overlooked in conservation planning initiatives. This is particularly true of ectomycorrhizal hypogeous fungi as there is a lack of information available about species distributions and abundance. This paper presents the culmination of two years of hypogeous fungi data collection carried out in the Wet Tropics of Far North Queensland, Australia. Hypogeous fungi were surveyed, using the time-standardised raking technique, four times per year in the early wet, late wet, early dry and late dry seasons. There was significant seasonality of total sporocarp numbers per hectare in the first year of data collection ($^{-}2 = 207.298$; df =3; $P < 0.001$). The abundance of sporocarps per hectare was relatively stable during the early dry (145.83 ha-1), early wet (137.5 ha-1) and late wet (240 ha-1) but dropped considerably to 6.67 ha-1 in the late dry season. There was a strong correlation between precipitation and sporocarp abundance, with rainfall in the first year following the typical seasonal pattern according to 74-year monthly means. The relationship was confirmed in the second year of data collection, when 'out of season' rainfall correlated with increased yield of sporocarps. Although the association between precipitation and hypogeous fungi sporocarp production has long been suspected, this is the first time this correlation has been confirmed. The implications associated with decreased production of sporocarps due to more frequent and prolonged drought periods that are expected with global climate-change, may include a reduction in biodiversity and changes to the distribution of hypogeous fungal species in tropical ecosystems. A reduction in biodiversity and availability of hypogeous fungi will in turn impact on mycophagous mammal dispersers and total ectomycorrhizal forest health.

PS5-540-0482

Leaf litter and leaf age as factors affecting the assemblage of endophytes associated with particular hosts.

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Extensive surveys across several host plant species suggest that different hosts appear to show relative abundance differences with respect to which fungi dominate them: those that are commonly found in association with one host often are either rare or absent in others. Furthermore, several studies indicate that, with time, the initially endophyte-free leaf tissues become saturated with endophytes, such that the proportion of sampled leaf fragments with culturable fungal isolates approaches or reaches 100 percent. However, available data from studies in which cohorts of newly flushed leaves are followed through time suggests that the diversity of endophytes usually declines as leaves age. Particular importance has been given of to the effect of forest canopy on enhancing the rate of initial endophyte colonization of seedlings. Here we compare the rate of endophyte accumulation when endophyte-free seedlings are placed under intact forest canopies with intact or removed leaf litter. The results show a much more rapid accumulation of endophytes in the presence of intact leaf litter. This not only suggests that dead leaves appear to be a primary source of inoculum of foliar endophytic fungi (FEF), it further suggests that local FEF sources (i.e., the local litter) can dominate the composition of colonizing spores. If true, this would provide one potential mechanism explaining reports of relatively fine scale local differentiation of FEF communities within the same host plant.

PS5-541-0496

Oak (*Quercus petraea*) leaf litter degradation by litter-decomposing fungi

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Sessile oak *(Quercus petraea)*, also known as Durmast oak, is a large broad leaf tree native in Europe and Asia Minor. It has also been widely planted in Europe to produce durable long lasting heartwood. Fallen oak leaves are rich in tannic acid and phenolic compounds. Thus their decomposition is troublesome e.g. in compost environments. Several species of litter-decomposing fungi (LDF) are specialized to degrade oak leaves using a manifold set of cellulolytic and ligninolytic enzymes.
Three oak-litter inhabiting basidiomycetous LDF species, namely *Mycena inclinata*, *Marasmius quercophilus* and *Pholiota lenta*, were used in an oak-leaf degradation study. The activities of their major extracellular hydrolases and oxidoreductases were recorded during a 12 week decomposition experiment. Analytical pyrolysis was used to examine the lignin composition changes.
Results suggest that the tested fungi used different approaches or sets of enzymes for the degradation of oak leaves to gain carbon from them. Rather high amounts of endo-1,4-beta-xylanase were recorded for all three species. They differed, however, clearly in the production of other enzymes. *M. inclinata* produced high amounts of laccase activity, P. lenta notable amounts of endo-1,4-glucanase and 1,4-beta-glucosidase, whereas for *M. quercophilus* equal activities of laccase, glucosidase and xylanase were found. Analytical pyrolysis showed a decrease of lignin/carbohydrate and syringyl/guaiacyl lignin ratios in decayed oak leaves. *M. inclinata* and *M. quercophilus* also caused a significant decrease of lignin content accompanied with the accumulation of syringic and vanillic acid, which is typical for wood decayed by wood-inhabiting white-rot basidiomycetes. Changes of pyrolysis profiles by P. lenta were less pronounced.
In conclusion, the utilization of oak leaves by LDF as growth substrate is possible with different sets of extracellular enzymes. There seems to be a dominance of xylanase among endohydrolases and laccase among ligninolytic enzymes.

PS5-542-0502

A List of Fungi Recorded in Japan

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Four books have so far been published in each of which fungi observed and/or isolated in Japan were listed . The first one was "A List of Japanese Fungi Hitherto Known" which was compiled by Shirai in 1905 and described about 1200 species. The second book, "A List of Japanese Fungi Hitherto Known 2nd ed." was published by Shirai and Miyake in 1917 and showed about 3500 species. The third one entitled "A List of Japanese Fungi Hitherto Known, revised and enlarged 3rd ed." edited by Shirai and Hara was published in 1927 and about 4500 species were recorded. The last one was "A List of Japanese Fungi Hitherto Known" which was published by Hara in 1954 and about 7000 species were included. Now, a new book entitled "A List of Fungi recorded in Japan", which has been initiated and compiled by K. Katumoto and edited by K. Ando, is being planned for publication. In the book, fungi recorded in Japan by 2005 will be listed. Moreover, books, journals and year in which the fungi were recorded in Japan, as well as their synonyms, hosts or substrata and any additional information concerning them will be described in the book. At present, 2396 genera and 12329 species have been listed up as shown below.

PROTISTS		12 genera	21 species
CHROMISTA	HYPHOCHYTRIOMYCOTA	1 genera	2 species
	LABYRINTHULOMYCOTA	3 genera	5 species
	OOMYCOTA	53 genera	362 species
FUNGI	CHYTRIDIOMYCOTA	32 genera	138 species
	ZYGOMYCOTA	86 genera	278 species
	ASCOMYCOTA	787 genera	3240 species
	BASIDIOMYCOTA	624 genera	4233 species
	Anamorphic fungi	798 genera	4050 species
TOTAL		**2396 genera**	**12329 species**

PS5-543-0514

Global and local genetic diversity of arbuscular mycorrhizal fungi

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Arbuscular mycorrhiza is believed to represent an ancient association between plants and fungi, and seems to have evolved with the first land plants. Several AMF species are cosmopolitans to be found from the Northern to the Southern hemisphere. The fungi are considered to have limited ability to spread between continents, which have founded the hypothesis that the fungi were dispersed before the continents detached 200 mill yr ago. This isolation should have lead to a considerable genetic diversification among AMF. This hypothesis was studied by analysing genetic diversity of three AMF species. Most molecular data available on genetic diversity of AMF represent ribosomal genes, which can be difficult to interpret as the AMF may hold a within spore gene diversity. In this study information from ribosomal genes were combined with analysis of protein coding genes and intron regions to provide reproducible data on genetic diversity. The analyses indicate that in spite of long genetic isolation of the fungi it is difficult to separate genetic variation at a local scale from variation at a global scale. This result can partly be explained by a long asexual evolution in the fungi.

PS5-544-0515

Will conservation based on vascular plants be sufficient for macrofungi and mosses – a Tasmanian study.

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Conservation and management decisions are currently based on vascular plant data; usually at the level of vegetation type. With little data on the distribution of macrofungi and mosses in Tasmanian vegetation, these cryptogams are rarely considered by conservation and management processes. Plants are currently being used as an untested, default surrogate management tool for conservation of macrofungi and mosses. To investigate this gap in understanding four Tasmanian vascular plant communities were used to assess the congruence of macrofungi and moss assemblages. These included wet forest, grassy woodland, coastal heath and alpine heath near Hobart.
Surveys of vascular plants, macrofungi and mosses were carried out at 32 sites from 1999, 2001-2003. Multivariate analyses (ordination and mantel tests based on presence/absence data sets) were used to assess congruence of taxon assemblages across vegetation types.
Ordinations and mantel tests show clear significant relationships within vegetation type between plants, macrofungi and mosses. Pearson correlation was the strongest between plants-macrofungi ($r = 0.686$) and between plants-mosses ($r = 0.575$). Both correlations are significant at $p < 0.001$. These strong correlations suggest that vegetation types based on knowledge of vascular plants may be a successful surrogate for common macrofungi and mosses within these communities. Capacity for vascular plants to be a surrogate for macrofungi and mosses when choosing conservation priorities will be explored using minimum set analyses.

PS5-545-0516

Fungal diversity of the islands on the Yellow Sea of Korea

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Fungi can live in almost any environment such as soil, sea water, or fresh water and inhabit plants and animals. Fungal ecologists try to figure out fungal diversities in different ecosystems using various methods. The Yellow Sea (West Sea) of Korea contains a number of small islands and is bounded by China. To study the fungal diversity of sea islands, we chose three islands that are at a geographic distance each other, surveyed and collected samples in spring, summer, and autumn three times a year. Without using culture-based techniques, we applied several methods including molecular cloning and signal molecules screening. From island soils, total genomic DNA was extracted and approximately 500 isolates were cloned from each island and used in fingerprinting the fungal community by amplified ribosomal intergenic spacer analysis (ARISA), amplified rDNA restriction analysis (ARDRA), and DNA sequencing. We used two kinds of signal molecules (ergosterol and glomalin) to analyze the biomass of island soils and compared collected mushrooms with those from Ulleung-do Island that lies on the East Sea of Korea. From the ARISA test, each island sample developed a seasonally different amplified pattern, but the ARDRA test didn't present so much differences. The ergosterol concentration was higher in summer than in other seasons and likewise the glomalin concentration showed a similar pattern. Each island showed different vegetation, meteorology, and geography, which might continuously induce differences of the fungal community structure, community development, and species diversity in compact island ecosystems.

PS5-547-0525

Conservation and utilization of fungal biodiversity at BIOTEC Culture Collection (BCC) Thailand

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BIOTEC Culture Collection is a specialized collection formally established in 1996 under the National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA). The principle objective are to provide safe deposition of cultures for researchers, to preserve and maintain microorganisms isolated from Thailand for future use, and to manage strain data using a standard system. Another major role is to supply cultures and related information for an in-house screening program for drug discovery from microorganisms.
The collection comprises 15,117 isolates of fungi from various sources and habitats: insect pathogenic fungi (3,833), freshwater and marine fungi (1,877), rice blast pathogenic fungi (1,148), soil fungi including those isolated from leaf litter and humus (975), wood decaying fungi including basidiomycetes (2,033), seed fungi (437), endophytic fungi (1853), lichenized fungi (383) and taxa from other sources (2,578). The collection also holds bacteria including actinomycetes (2,710) and yeasts (1,081).
Preservation methods used are freezing at -80oC and -170oC (in a vapor phase of nitrogen), freeze-drying and under liquid paraffin. Strain data are managed by using a computer program called Microbial Information Management Systems (MIMS). e-BCC is a program connected to MIMS used to publish catalogue of strains through the internet. On-line catalogue is available at http://bcc.biotec.or.th/

PS5-548-0531

Macrolichen diversity in relation to diversity of woody plants

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In studies concerning nature conservation issues common lichen species have usually been neglected although collecting of these results gives comparatively small disturbance of the populations and is easily done. Instead rare or threatened species or species usually have been used as indicators of sites with high biodiversity.
Here, the macrolichen diversity is compared with the diversity of woody plants and other characteristics of different sites in Estonia, Finland and Sweden as a part of a larger project including comparative studies on habitats with presumably high species diversity The site selection was based on the occurrence of *Daphne mezereum* which usually occurs in semi-open habitats in transitions zones containing species from the surrounding biotopes. One of the main objectives with the study was to develop a fairly rapid method of evaluation of biodiversity using easily identified species. As total inventories are time consuming and reflects snapshots of a certain occasion it is beneficial to use other methods which may give a little less but sufficient information for many purposes, e.g., estimations on biodiversity. The ecological and evolutionary processes that shape diversity and distributions are general and results are assumed to be translatable from the target species to other species. The combination of data from a small number of species may constitute a useful monitoring protocol for lichens and higher plants.
In total about 50 lichen species and 25 substrates are included and analyzed in the study. Most of the most common lichens are sorediate or isidiate and asexually reproducing and occur on several substrates. The relation between the diversity of lichen and woody plants is presented.

PS5-549-0536

Assemblages of endophytic fungi on Salicornia europaea

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Salicornia europaea (Chenopodiaceae) is a halophytic succulent plant widely distributed in salt marshes in the northern hemisphere. In Japan, distribution of the plant is geographically split into two locations; eastern coast of Hokkkaido and the Inland Sea region. Salicornia europaea is known to have originated from Hokkaido in Japan, and it is considered that the plant seeds were transferred with ballast sands from Hokkaido to Inland Sea by the boats collectively called "Kitamae-bune" that were mainly used for salt-transportation between the two regions in the middle Edo era (18 century). In this study, we investigated assemblages of endophytes of the plant distributing in the two such drastically separated areas to reveal ubiquitousness and host-preference of endophytic fungi growing on S. europaea. Plant samples were collected near Lakes Notoro and Saroma (ca. 44N, 144E) located along the Sea of Okhotsk, and from the sites of salt pan in Okayama and Kagawa Prefectures (ca. 34N, 133E). Fungi were isolated from aerial parts of the plant and seeds after surface sterilization using sodium hypochlorite followed by incubation on cornmeal seawater agar medium. As a result, *Stemphylium* spp. were isolated with a colonization frequency (CF) as high as 60% from aerial parts of the plant collected near Lake Notoro. Subsequently, other dematiaceous fungi such as *Alternaria* spp. (35% CF) and *Pleospora* spp. (20%, teleomorphic) were also isolated. From the plant samples collected near Lake Saroma, *Alternaria* spp. (60%) were isolated most frequently, but *Stemphylium, Pleospora,* and *Cladosporium* spp. did not appear at high frequencies. From the sites in the Inland Sea region, Alternaria (60-100%), *Pleospora* (40-80%), and *Stemphylium* spp. (15-30%) were isolated frequently. These results indicate that dematiaceous fungi were major endophytes on *S. europaea*. Similar results have been reported in other studies on endophytes of chenopodiaceous halophytes in England and Canada. Dematiaceous fungi found from aerial tissues of various terrestrial plants have been generally recognized as epiphytes that penetrate tissues as their hosts become old. However, the present study revealed that some of the fungi are able to penetrate even fresh tissues of halophytes growing in salt marshes and live as their major endophytes worldwide. On the other hand, no fungi were detected from the seeds of *S. europaea* growing at a site in Okayama Pref, which suggests that the endophytes found from aerial parts were not likely to be transferred vertically through seeds. Thus, the similar assemblage pattern of the endophytes of *S. europaea* observed at locations that are far apart from each other may be due to the host-preference of the dematiaceous fungi.

PS5-550-0539

Macrofungal diversity, variation in time and space in tropical lowland forests in the Colombian Amazon

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Knowledge of fungal diversity has increased during the last years in Colombia. However, many of the fungal explorations were local inventories, new species descriptions, or new records for the country. In addition, most of these studies were carried out in montane forests.
Here, we made an attempt to study the effect of reforestation time and different landscape units on macrofungal communities. For this goal, 4 different plots located in Amacayacu National Park (Colombia), were established. Two of them in flooded forests and two in Terra Firme forest.
The macrofungal survey was performed between August 2003 and October 2005 and comprised six visits. In total 524 collections were made in all the plots. Approximateely 218 morpho-species belonging to 50 families were identified. The family Marasmiaceae was collected most frequently, with *Marasmius* as the most abundantly collected genus.
The highest number of species, as well as the highest number of collections, occurred in the flooded forest on the Island (n=85), followed by the flooded forest on the mainland (n=83). For those located in Terra Firme forest, the number of species was quite similar in both plots (namely, 68 and 65 morpho-species, respectively). The Jaccard index showed a conservative similarity of macrofungal composition for the plots located in flooded places, as well as for those occurring in Terra Firme forests. This pattern was similar to that observed for the higher plant species.
The differences observed, either in time of collection as well as between the plots, could be explained by the spatial distance between the plots and the effect of environmental processes in time. The former is relevant when the distances in the different landscapes are not too big. On the other hand, regional and seasonal differences in rainfall and flooding may create a mosaic of substrates and environments affecting the fungal community structure.

PS5-551-0541

Macromycetes from the middle Caquetá region (Colombia, Amazon)

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Three hundred and thirty six collections of macromycetes were examined from eight localities in the middle Caquetá region in the Colombian Amazon. The collections were made during the following projects: 1. "The role of fungi in the regeneration of Colombian Amazon forests" (NWO/WOTRO WB 84-525) in which mixed lowland forests as well as a mixed forest dominated by *Pseudomonotes tropenbosii* (Dipterocarpaceae) were sampled over a four-year period; 2. "Ethnobiological study of the macromycetes by the Uitoto community of Araracuara region", which focused on the use and handling of fungi by Uitoto natives. Collections were kept in the Herbarium of the University of Antioquia (HUA) and the Colombian National Herbarium (COL). The middle Caquetá region comprises approximately one million ha. of primary tropical forest, corresponding to the Tropical Rainforest life zone (Bh-T) according to the Holdridge system, as well as some areas of regeneration forest resulting from traditional agricultural culture by the natives.
From all the collections revised, 133 species were identified, from which 14 belong to the Phylum Ascomycota, distributed in 11 genera, 5 families and 4 orders, and 119 species belong to the Phylum Basidiomycota, distributed in 66 genera, 30 families and 18 orders. From these, one corresponds to a new species, 22 are new records from the Amazonas department, 23 species are new records from the Caquetá department and 57 species are new records from Colombia. The greatest number of genera and species found were from the families Xylariaceae (Ascomycota), Tricholomataceae and Coriolaceae (Basidiomycota).
Decomposing wood is the most commonly used substrate. Ectomycorrizal species, such as Austroboletus sp. nov. (Boletaceae), were founded mainly in the mixed forest dominated by Pseudomonotes tropenbosii (Dipterocarpaceae).

PS5-553-0543

Ethnomicological studies among indigenous Uitoto from Colombia Amazon

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In spite of ongoing processes of cultural changes that have affected the indigenous and farmers groups of Colombia, these still have knowledge on the traditional use of the natural environment. This has been the result of a continuous experimentation allowing them to survive and to adapt to the hazardous and difficult-to-survive environments of the wild.
The Uitoto indigenous live widely distributed in the Colombian Amazon. For this work we visited three indigenous communities settled along the middle Caquetá river, Departments of Caquetá and Amazonas, Colombia.
For the Uitoto natives, the fungi originated from the body of the God. He 'drew them out little by little', 'placed them in nature', 'gave names' and 'used some of them'. Due to the idea that the fungi may act as illness agents, the total of species 'purified by the creator' comprises only 11 species, of which eight are used for consumption, and three are used in medicine.
Other fungi, related to the mythical cosmological vision of the Uitoto, exist, such as the 'true fungus' (*Phellinus* sp. and *Ganoderma* sp.) and hallucinogenic fungi, such as *Psilocybe cubensis* and *Panaeolus antillarum*. The Uitoto people practice a classification system according to the use of fungi, in which they recognize four groups: edible, medicinal, magical and the rest of species that are not used.
The fungal consumption relates to times of hunting shortage, which usually is the time of heavy rainfall in the wet season, during which the carpophores grow abundantly on decomposing wood in the traditional farming zones (i.e. chagras).

PS5-553-0544

Biodiversity of marine fungi from mangrove areas in Malaysia

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Six locations in Malaysia have been studied for marine fungal diversity from 1994 to 2004. The study sites were Kuala Selangor, Morib, Port Dickson, Pantai Remis, Langkawi Island (Peninsular Malaysia) and Gaya Island (Sabah) where collection was randomly collected from attached and drift decaying wood materials of intertidal mangrove areas. This study was conducted to study biodiversity of manglicolous fungi in Malaysian mangrove and to investigate the richness of marine fungi in mangrove ecosystem in Malaysia. Two hundred twenty nine species of marine fungi were recorded from five thousand one hundred eight total samples collected with only 84.4% of wood colonized by these fungi. Ascomycota (192) was abundant in the mangrove ecosystem of Kuala Selangor, Morib, Port Dickson, Pantai Remis, Langkawi Island and Gaya Island followed by Deuteromycota (35) and Basidiomycota (2). Species frequently encountered were *Lignincola leaves* Höhnk (17.9), *Verruculina enalia* Kohlm. & Volkm.-Kohlm (13.9%) *Trichocladium achrasporum* (Meyers & R. T. Moore) Dixon (12.9%), *Savoryella lignincola* E. B. G. Jones & Eaton (12.4%), Dictyosporum pelagicum (Linder) G. C. Hughes (11.9%), *Lulworthia grandispora* Meyers (11.5%), *Halocyphina villosa* Kohlm & E. Kohlm (11.6%) and *Periconia prolifica* Anastasiou (10.1%). The average number of fungi per sample was 2.88. Thus, this study summarized the diversity of marine fungi collected from Malaysian mangroves.

PS5-554-0553

Digital libraries for systematic mycology

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Free and easy access to publications is important in all branches of science. In systematic mycology, many of the most important publications are rare, old and present only in larger libraries mainly in Europe and North America. For mycologists in most of the rest of the world consulting those works is difficult and time-consuming - a severe impediment to any biodiversity work. A new co-operative and collaborative initiative aims to address this problem by making specialist bibliographic information (older references, cyrillic literature etc.) and scanned images of key publications freely available on the internet. In the first phase, the following items have been prioritized:
the main catalogues of fungal names (Saccardo's Sylloge, Zahlbruckner's Catalogus, Petrak's Lists, Index of Fungi);
the volumes containing lists of bibliographic references to mycological works (Lindau & Sydow's Thesaurus & Supplement, Ciferri's Supplement, Bibliography of Systematic Mycology);
the sanctioning works (Persoon's Synopsis, Fries' Systema etc.);
Searchable bibliographic references to over 59,000 works.
By the end of 2006, all of these for the 19th and 20th centuries will be freely available on-line, with significant integration between those images and names in Index Fungorum (http://www.indexfungorum.org). In the second phase, with the kind consents of their respective publishers, all except recent issues of Mycotaxon and Annales Mycologici / Sydowia will gradually become available, with the aim of digitizing all material of those volumes for which the publishers' consents have been given by the end of 2007. Other works are also being added on an ad-hoc basis, with both volumes of Michelia, and significant amounts of Grevillea and of the publications by Spegazzini already available on-line: already over scanned images of over 50,000 pages are available. For more information, please visit the Cyberliber (http://www.cybertruffle.org.uk/cyberliber/index.htm) and Libri Fungorum (http://www.librifungorum.org) websites.

PS5-555-0560

Prescribed Fire And Wood Inhabiting Fungi In A Pinus sylvestris Forest

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Many boreal organisms are more or less associated with fire perturbation. However, specific knowledge on the fire dependence among particular species is scarce. This is true for most species groups including wood-inhabiting fungi. In Sweden, forest companies and county administrations have started to use fire as a management option in forest reserves. Therefore, it is essential to evaluate prescribed burnings and gain information about species responses on forest fires. In this study, wood-inhabiting fungi (polypores and corticoids) were examined after a prescribed fire. We asked; how does fire affect wood inhabiting fungi and how is the colonization process? The study site consists of two 30 ha *Pinus sylvestris* dominated forest sites (500m apart). One area was burned in 2001 while the other was left as a reference and control area. In September 2000, 2001, 2003 and 2005, 323 logs were studied, 155 in the fire area and 168 in the control area. The logs were individually numbered, measured and scored for fungi. The fire negatively affected several of the wood-inhabiting species and the total species richness in the fire area decreased from 71 to 50 during the three first years. Fruit bodies of *Hyphdontia hastata*, *Junghunia luteoalba*, *Skeletocutis lenis, Tubulicrinis spp.* decreased or disappeared. A number of species showed a positive response, namely *Athelia* spp., *Botryobasidium obtusisporum*, *Galzinia incrustans* and the red-listed *Dichmitus squalens* (EN). Results so far indicate that a number of species benefit form fire and that some species seems to be fire dependent. The study shows that prescribed burning seems to play an important role for maintaining species richness of wood inhabiting fungi.

PS7-514-0561

Poroid Mushrooms For Pulp Production Processes

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Production of pulp from wood is a relatively great energy consuming process and wastes that are produced during the production processes become a source of environmental pollutions. In nature, there are numerous types of fungi known as wood degraders, which degrade the wood components at different capacity. The lignin-degrading fungi that degrade lignin component of wood may or may not attack cellulose. Fungi that are good as lignin degraders but poor in utilize cellulose should be benefiting if they are used in pulp production industry. Isolates of 113 poroid mushrooms, collected from tropical rainforest were screened to determine their potential use to degrade lignin materials from wood in pulp production. Evaluation on the isolates degradation ability began with qualitative detection for cellulolytic activity. Of all the tested fungi, 70 isolates showed relatively low or no cellulolytic activity. Then, when these fungi were grown on media for the qualitative ligninases detection, 19 isolates showed having relatively high rates of the enzymes activity. Quantitative assay on enzymes activities were also determined. The results indicated that cellulases activity were in a range of 1.6-6.7X10-2 Uml-1 day-1. Ligninases activity assay in the 19 isolates indicated the present of laccase activity, in a range of 2.5x10-3 -1.04x10-2 Uml-1day-1; however no activity of lignin peroxidase or manganese peroxides were detected. Degradation tests on Acasia mangium wood chips showed the reduction of the chips weight after two weeks inoculated with the fungi isolates were 24.3% to 43.8%. Amongst the isolates that reduced the chips weight at the high percentage were of *Trametes versicolor, Ganoderma sp, Pycnoporus coccineus*, *Haxagonia* sp., *Phellinus* sp., *Microporus* sp. and *Fomes* sp

PS5-556-0574

Scolytodes unipunctatus (Coleoptera: Scolytinae): a new lineage of ambrosia beetles with a unique spectrum of nutritional fungi

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Ambrosia fungi are strictly entomochoric and mutualist associates of ambrosia beetles (Coleoptera: Scolytinae). We studied mycobiota associated with Scolytodes unipunctatus infesting Cecropia trees in Costa Rica. Isolates were examined using morphology and phylogenetically placed using rDNA (ITS region, LSU and SSU rDNA). All principal associates belong to ophiostomatoid fungi and are essentially distinguished from the other known ascomycetes. New species of the genus *Ambrosiella* (Ophiostomatales) was found bearing nutrient rich exudates on the tips of the vegetative hyphae. The exudates, which are enclosed in a gelatinous capsule, are probably an adaptation to ambrosia lifestyle. Another two species belonged to the genus *Gondwanomyces* (Microascales). The genus was originally known from *Protea* inflorescence from South Africa only, but its distribution and ecological niche can be broader. The last fungus phylogenetically close to the genus *Graphium* (Microascales) posessed only a *Scedosporium* anamorph, while lacking synnematous synanamorph. This species also produces a typical ambrosial growth stage ("sprout cells") and should be probably placed to a new genus, near to *Graphium*. The finding of a niche with many unique fungal taxa is congruent with taxonomical isolation of their insect vector suggesting their coevolution.

PS5-557-0578

Geosmithia fungi are highly diverse and consistent associates of bark beetles: a proof from their host preference in temperate Europe

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Geosmithia (Ascomycota) are rather unknown dry-spored *Penicillium*-like fungi, which occur in galleries of many phloeophagous bark beetles. We collected wood samples infested with 22 bark beetle species from temperate Europe. *Geosmithia* isolates from beetles were sorted to 17 operational taxonomic units (OTUs), each with a unique RAPD pattern, phenotype and with common evolution of ITS region. The OTUs represent 6 known species and 10 yet undescribed taxa. Ninety-two samples infested with subcortical insects were characterized by presence/absence of OTUs and the similarity among them was evaluated. Non-random distribution of *Geosmithia* OTUs was found that correlated with the substrate (vector and host plant) but not with the location. Different populations of the same beetle species transmit relatively constant *Geosmithia spp*. communities in large geographic area. OTUs pattern of each vector is shaped by the degree of the spatio-temporal isolation of their niches. Thus, *Geosmithia* assemblages on sympatric bark beetle species are highly similar. The similarity decreases within the groups of facultative sympatric species and among different species of allopatric bark beetles, a pattern resembling that of ophiostomatoid fungi. This suggests that spore type is not important to primary establishment of insect-fungus association. Ancient origin and long-term consistency of symbiosis between *Geosmithia* and bark beetles is suggested.

PS5-558-0594

Phytophthora species in forest ecosystems of western Nepal

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Phytophthora ramorum is an invasive pathogen in Europe and western North America on many ornamental shrubs and forest trees; *P. kernoviae* sp. nov. is a similar invasive so far confined to the UK. Asia is a centre of diversity for many plant genera that are hosts to the two *Phytopthoras.* The pathogens are hypothesised to have originated in Asia, such as Yunnan, Taiwan or the Himalayas, and transferred via commercial movement of plants in the rest of the world.
To investigate whether the pathogens might be is present in Himalayan forest ecosystems an expedition was organised in October 2006 in the Western Nepal. An intensive soil sample survey was conducted during a round trip from Kolti (1396m) to Rara Lake (3500m). The geographic co-ordinates of the samples were recorded by GPS and used to construct a distribution map.
47 soil samples were collected in the rhizosphere of 17 target broadleaved and coniferous trees and shrubs. These were collected from three distinct ecological zones: temperate forest, sub-tropical forest and sub-tropical agriculture. Samples were baited using three different methods and the bait material plated onto a selective medium. Resulting colonies were identified on the base of their morphological, behavioural and molecular traits. A total of 39 *Pythium* and 89 *Phytophthora* isolates were obtained. Three *Phytophthora* species were present but neither P. ramorum nor P. kernoviae were isolated.
P. citricola was the most frequently isolated species, and was confined to the soil around temperate forest trees including Acer, Aesculus, Juglans, Ulmus and Vibernum. A distinct species of *Phytophthora*, with some similarities to *P. meadii* was associated with the sub-tropical forest vegetation including Lithocarpus, Cupressus, Cornus, Carpinus and Castanopsis. This may be a previously unknown species. A third species, *P. palmivora* was only found in the sub-tropical agricultural zone around Ficus, Persea and Olea.
Although *P. ramorum* and *P. kernoviae* were not found, the distinct zoning of the two possibly endemic forest *Phytopthoras, P. citricola* and the *P. meadii*-like *Phytophthora*, may lead to further understanding of the role of the genus in forest ecosystems. Whether the *P. meadii*-like *Phytophthora* may present a threat to other forest ecosystems has yet to be determined. The discovery of *P. palmivora* in association with native Olea trees in Nepal may have significance for proposed commercial cultivation of Olea species in this region and elsewhere.

PS5-559-0596

A european red list for larger fungi in progress

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There is good scientific evidence that populations of many fungi are declining in Europe. In order to attract interests and to initiate conservation actions A European-level Red List for larger fungi is of pressing importance. The European Council for the Conservation of Fungi (ECCF) under the body of the European Mycological Association (EMA) has thus started the production of a European Red List for larger fungi according to criteria of IUCN. It is planned to be completed by the end of 2008.
The mycological knowledge in Europe is comparable satisfactory in comparison to other regions of the world despite at least 15 000 taxa of larger fungi is known. In fact, at least 27 European countries already have national fungal Red Lists. Sufficient information, expertise, infrastructure and interest exist to make production of a European Red List practical.
The red-listing will be an open process largely using internet, based on information of occurrence, ecology and status of species from national mycologist, compiled and analysed by an European expert committee and exposed for review. The pragmatic approach taken is that all national fungal Red Lists are being compiled and supplemented with additional proposed species. This gross list, currently containing more than 5000 taxa, is being processed and reduced by experts before request for national information. The poster will present the aims, the plans and the programed outcome of the project.
Red Lists are important instruments in both national and international conservation work as they are catalogues of species whose future survival is uncertain and presents an analysis of the relative risk of extinction faced by the species inhabiting a certain area, e.g. a country, a continent or the world.

PS5-560-0598

Ophiostomatoid fungi associated with the spruce bark beetle Pityogenes chalcographus in Austria

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The phloem-feeding bark beetle *Pityogenes chalcographus* is an economically important forest pest in Europe. This insect primarily infests Norway spruce *(Picea abies)* and occasionally other conifers. Most conifer bark beetles live in symbiosis with blue-stain fungi, belonging to the ascomycete genera *Ophiostoma, Ceratocystiopsis* and *Ceratocystis* and to related anamorph genera such as *Leptographium* and *Pesotum*. They are also known as the ophiostomatoid fungi. Many ophiostomatoid fungi associated with bark beetles cause discoloration of the sapwood of conifers and some species are pathogenic to their host trees. The aim of this study was to investigate the species spectrum and abundance of ophiostomatoid fungi associated with *P. chalcographus* in Austria.
Stem sections of Norway spruce colonized by *P. chalcographus* were collected in 1993 and from 1996-1999 at various localities in Austria. Malt extract agar (2 % malt, 1.6 % agar, supplemented with 100 mgl-1 streptomycin sulphate) was used as medium for isolations. Fungi were isolated directly from the beetles as well as from the phloem and sapwood of beetle-infested trees. Isolations were also made from phloem and sapwood tissues taken from logs that had been inoculated with beetles five to six weeks earlier. Additionally, a few isolates were obtained from conidia and ascospores taken from fungal structures occurring in the galleries of the insects. Ophiostomatoid fungi were identified based on morphological characteristics of sexual and asexual stages.
In total 11 ophiostomatoid fungi were found to be associated with *P. chalcographus* in Austria. Ophiostoma ainoae, a *Leptographium* sp. and a *Pesotum* sp. were most frequently isolated, and *Ceratocystiopsis minuta, Graphium fimbriisporum* and *Ophiostoma piceaperdum* were also relatively common. Rare components of the ophiostomatoid mycobiota of *P. chalcographus* included *Ceratocystis polonica, Ophiostoma bicolor, O. cucullatum, O. floccosum* and *O. piceae*. *Ophiostoma penicillatum*, previously reported as fungal associate of *P. chalcographus* was not found in this study. The *Leptographium* species commonly associated with P. chalcographus appears to represent a new taxon, based on morphological and rDNA ITS sequence comparisons. The virulent blue-stain fungus, *C. polonica* was only rarely recorded as fungal associate of *P. chalcographus*. This is in contrast to Ips typographus, the most economically important spruce bark beetle species in Europe, which is a common vector of *C. polonica*. In conlusion, *P. chalcographus* is a consistent vector of a diverse assemblage of ophiostomatoid fungi in Austria, but the associated fungi likely display only moderate or low levels of virulence to Norway spruce.

PS5-561-0611

Sixteen rust fungi from Northeast of Iran

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The field survey of the Golestan Province, northeast of Iran, in the past 10 years resulted in the new geographic distribution and host records of sixteen rust fungi in Iran: *Melampsora coleosporioides* Dietel , *M. allii- populina* Kleb., *Phragmidium bulbosum*(Strauss) Schlechtend , *Ph. violaceaum* (Schultz) Winter, *Ph. rosae-pimpinellifoliae* Dietel , Ph. tuberculatum Muller, *Puccinia absinthii* (Hedw.f.)DC. , *Pu. allii* (DC.) Rud., *Pu. coronata* Corda f.sp. avenea Erikss. , *Pu. coronata Corda* f.sp. hordei Jin and Steffenson , *Pu. graminis* Pers.:Perss.f.sp. avenae Eriks. and Henn. , *Pu. hordei* Otth. , *Pu. persistens* Plow. subsp. triticina (Erikss.) Urban et Markova , *Tranzschelia discolor* (Fuckel) Tranzschel and Litv. , *Uromyces striatus* Schroet and U. viciae-fabae(Pers.) Schroet. *M. coleosporioides* and Ph. *bulbosum* are new for the Iranian mycoflora. Pu. *coronata* f.sp. *hordei* is new to Iran and probably Asian barley mycoflora.

PS5-562-0635

Effect of Toxic Metals on Gene Expression of Fungi in Relation with Bioremediation

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Bioremediation of toxic metals such as chromium (Cr6+) and cadmium (Cd2+), radionuclide ion uranium (U2+) is very important for a safe environment, human and animal health, and security. Despite scientific efforts, detection of these metals at low levels has been difficult. Therefore, the objective of the denoted study was to use soil-borne fungus Aspergillus flavus as biosensors of these metals. The interactive effect of the soil-borne Aspergillus flavus with toxic metals such as Cr6+, Cd2+ , and radionuclide (U2+) ions was studied at the cellular and molecular level. The interactive effect was assessed through fungal growth and gene expression. The response of soil-borne fungi to toxic heavy metals is important, since this organisms spends great part of its life cycle in the soil, especially in polluted sites and presumably participating in soil bioremediation processes. Fungal cultures were independently treated with metal ions as salt sulfates including Cr6+, or Cd2+ , or U2+ acetate, at 5 ppm concentration. Fungal cultures were assayed for growth and total RNA, after 2, 4 or 8 days incubation. Fungal cultures showed the same ratio between RNA yield and fungal growth, 1.8 or 2.5 µg RNA/g of wet mycelium after 4 days and 8 days, respectively, when exposed to Cd2+ or Ur2+, while the control (cultures without metals) showed lower ratio. Gene expression in A. flavus culture in response to Chromium and Uranium were studied in RT-PCR experiments by using specific primers for aflatoxin production. The expression of protein-encoding loci for O-methyltransferase (omt) and calmodulin (cmd) involved in aflatoxin production. The metal-treated cultures showed enhanced gene expression, as compared with non-treated cultures. The gene expression was more enhanced in older cultures. There was also some homology between PCR products amplified and the cytochrome P450 monooxygenases that catalyze oxidative reactions, which are common in microbial biodegration processes. Fungal cultures without metals showed the lowest gene expression. The metals did not affect the fungal growth during the experiments. Uranium maintained the redox potential of the fungal cultures, while Chromium reduced the voltage of the culture to negative values.The results suggest possible usage of the A. flavus studied as biosensors in bioremediation of toxic and/or radioactive metals.
The research was supported by NATO grant.

PS5-563-0636

Constructing a structural and biochemical database for the Fungi

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As part of the Assembling the Fungal Tree of Life project a searchable database of selected subcellular and biochemical characters (aftol.umn.edu) has been created as a source of characters for use in phylogenetic analysis and character reconstruction. The database provides access to data from a variety of traits by taxon or character state. Most data have been derived from publications. Data can be retrieved as NEXUS files. The resolution of character states for three traits, septal pore apparatus, nuclear division, and spindle pole body cycle will be demonstrated using maximum parsimony on a summary cladogram of known phylogenetic relationships of the Fungi. Problems encountered in developing the database include uneven documentation of published data with cultures and/or specimens, incompletely illustrated characters, variable fixation quality in ultrastructural studies, and determination of homology for characters that change with life cycle stage and developmental state. Mycological community input is requested to develop the database to its full potential.

PS5-564-0643

Structures of some white polypores.

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The anatomical drawings of EJH Corner established a standard of representation based on living specimens. I was privileged to have prepared these hitherto unpublished drawings under his direction, inspiration and encouragement between 1950-54. This poster shows some of the microscopical and illustrative techniques he taught me to use in order to present fruit-body structure in three dimensions by reconstruction from camera lucida images of individually dissected out hyphae. The species studied are now placed in the separate distinct genera of Tyromyces, Oligoporus and Skeletocutis largely because of the application of Corner's principle of hyphal analysis.

PS5-565-0685

Etnomycology notes of eatable macromycetes in the Ecuador.

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Ecuador has a natural and ethnic wealth that become mega diverse and an interesting place to develop etnobiologist studies. Ethnics inside their customs include in their diet several macromycetes species, those represent a basic source for their nutrition. The field work was carried out among the years 2002 - 2005, in the communities of: Sierra. - Kichwas - otavaleños; Kichwas - Pintag and Colonists. Amazonía. -Kichwas, Shuaras and Secoyas. Costa. - Chachis and Colonists, where collections and informal surveys were made with indigenous and colonizers, describing preliminarily the etnomicological wealth of the main species of eatable mushrooms in Ecuador. The main species consumed were: *Agaricus bisporus* and *Coprinus comatus* (Kich-S/Col.) "Callambas" or "*Kiru Callambas*"; Colonos "Callambas de Finados" (Col.); *Cordyceps melolonthae* (Kich-A Sec./ Shuar) "Supay Chaqui Ala", "Huati uncu têti", "Sapi"; *Oudemansiella canarii* (Kich-A/Sec/Sh) "Chincha ala", "Imi tëti", "Huamp"; *Pleurotus sajor-caju y* P. djamor (Kich-A/Sec/Sh/Chac./ Cof./ Col. "Taka ala", "Ai_tëti", "Shushui esenp", "Anj kijtiutiu", "Tutu'ccucho tsina", "Española blanca";
Lentinus crinitus y L. badius (Kich-A /Sec/Sh) "Sara ala", "Taque nuti tëti", "Untushi"; *Auricularia delicata* y A. *fuscosuccinea* (Kich-A/Sec/Sh/Chac./ Col.) "Calulu ala", "Caro tëti", "Iwianchi kuishi", "Isk" or "Bulum kijtiutiu" and "Orejas de mono"); *Polyporus tenuiculus* (Kich-A/Sec/Sh/Col.) "Busun ala", "Po tëti", "Shushui esenp", "Pusunera"; *Schyzophyllum commune* (Kich-A) "Aya ala". *Pleurotus* and *Auricularia* are more frequent in "chacras" (sew area near the house). The studied species grow in dead woody material with the exception of *Agaricus, Coprinus* (humicolous) and *Cordyceps* (entomopathogen). Tales are developed about mushrooms ant their relation with the rain forest spirits and their fructification; it came with strong rains and rays. This information helps the community to classify the mushrooms. Women are the native experts in eatable mushrooms, based in their daily activities; as well as men in primary forest. The most common consumption is a kind of craft of leaves called mayto (Kich), cuaisu'u (Sec.), ayampaco (Sh.), pando (Chac.) and fried mushrooms or soup of mushrooms (Col.). Therefore, the use of eatable mushrooms contributes to the well-being of the community and their environment; and fragile ecosystems damages attempt against the ancestral knowledge of native people.

PS5-566-0686

A decade of rotten research in the wet sclerophyll forest of Tasmania

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Intensive forestry inevitably alters forest dynamics such as the processes of fungal and invertebrate succession in living trees and dead wood on the forest floor. The extent to which current silvicultural practices alter the elements of biodiversity associated with such processes in Tasmanian wet eucalypt forest is unknown and research is still in its infancy. Over the past decade we have sought to provide data with which to evaluate this issue in the wet sclerophyll *Eucalyptus obliqua* dominated forest of southern Tasmania, as well as identify key ecological processes, habitat types, and species at risk from certain forest practices.
In production coupes rotation lengths of around 80 years will eventually lead to the elimination of older trees and large diameter logs. Several large field studies have been undertaken to investigate this effect e.g. we destructively investigated whether small diameter logs (30-60cm) follow similar decomposition processes to large diameter logs (>100cm), and so support similar rot types, fungal and beetle assemblages; we felled living trees in each of three age classes (69, 105 and >150 years old) and examined the decay, related fungi and beetles within the main stem. We have accumulated a large collection of over 7000 cultures of fungi involved in wood decomposition of logs and standing trees. Morphological and molecular techniques are used to group isolates and match them to fruit bodies, to directly identify fungi from rotted wood and provide indicator species of late successional processes.
Our studies have shown the critical importance of retaining large diameter logs and old trees within the forest landscape in terms species richness and diversity for both fungi and insects e.g. the findings show that rot patterns and fungi in large diameter logs generally differ to those in small diameter logs. Beetle species which are characteristic of brown rot in large diameter logs are possibly poor dispersers and this invertebrate community and the fungal successional processes which result in this rot may be of particular conservation concern in production forests.
The research indicates that veteran trees and large diameter logs are important in providing continuity of habitat for the re-establishment of certain species following stand level disturbances, whether induced by logging or by wildfire. A degree of landscape-level planning in Tasmanian forestry is recommended that would maintain veteran trees and large diameter logs in the production forest landscape indefinitely.

PS5-567-0700

Microorganisms Section of NIAS (National Institute of Agrobiological Sciences) Genebank

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Microorganisms Section of NIAS Genebank (acronym; MAFF, URL; http://www.gene.affrc.go.jp/), established in 1985, contains various filamentous fungi, mushrooms, bacteria, viruses, protozoa and so on. These microorganisms are distributed to all over the world. We introduce activities of this section here.
1. Preservation and Distribution: This section maintains 22,253 microbial strains that are mainly preserved by cryopreservation, and is characterized by a wide collection of plant pathogens, e.g., Pyriculalia grisea, Fusarium species and edible mushrooms. The numbers of strains deposited and distributed amount to 800-1000 and 700-1,000 a year, respectively.
2. Exploration: This section has explored many areas within Japan [from Okinawa and the Bonin Islands (subtropical) to Hokkaido (subarctic)] for useful microorganism resources including phytopathogenic fungi, mushrooms and so on.
3. Characterization: In 2005, 6,992 characterizational data were accumulated. As kinds of characterization, there are phytopathogenicity, DNA sequences, genes, production of enzymes and toxins, salt tolerance, types and others. These data are input in a database, and some data were retrieved through the web site.
4. Publication: To promote utilization of the collections, Microorganism Genetic Resources Manuals are published every year. The latest issue (No. 18) is as for Pyriculalia grisea which is important in rice cultivation. The manuals including previous ones are available with PDF files on the web site. We also publish annual reports on exploration and activities in this section.
5. International Workshop in NIAS Genebank: Every 3 or 4 years, an international workshop is held, where studies of agricultural microorganisms are presented. The latest one was the 2004 Workshop, with the theme of "Diversity and Use of Agricultural Microorganisms", bringing together many researchers from Asia and USA.

PS5-568-0702

A Method of Long Term Preservation of *Phytophthora* and *Pythium* in Vapour Phase of Liquid Nitrogen

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Oomycetes is known to be difficult to preserve by cryopreservation. We tried to establish a cryopreservation technique of several species of *Phytophthora* and *Pythium* belonging to Oomycetes using strains which had been deposited in *Genebank of NIAS (National Institute of Agrobiological Sciences)*. *Seventeen strains of six species belonging to Phytophthora* (Ph. *cactorum*, Ph. *Citrophthora*, Ph. *Infestans,* Ph. *megasperma* var. *sojae*, Ph. *Nicotianae* and *Ph. vignae*) and 20 strains of 10 species belonging to *Pythium* (Py. *aphanidermatum*, Py. *debaryanum*, Py. *deliense, Py. echinulatum*, Py. *graminicola*, Py. *hydnosporum*, Py. *irregulare*, Py. *splendens*, Py. *Torulosum* and Py. *ultimum*), which had been deposited in Genebank of NIAS (National Institute of Agrobiological Sciences) but not preserved in liquid nitrogen, were tested for cryopreservation in vapour phase of liquid nitrogen. Five to ten disks (6 mm in diameter) of each strain were cut from mycelial lawn on a hemp seed agar plate were put in a plastic cryo-tube with a cryoprotectant consisted of 10% skim milk and 10% glycerol, and then the tubes were set in the freezing containers (Mr. Frosty, Nalgen Co. Ltd.) to be kept at 5 °C for 24 hr. Then the containers with the tubes were then kept at -70 °C for 2-5 days, and the tubes were transferred to vapour phase of liquid nitrogen (ca. -165 °C). One month or 5 years after preservation, the tubes were quickly thawed at 40-50 °C and then the thawed mycelial disks were tried to be cultured on Hemp seed agar plates at 20-25 °C. Thirteen strains of *5 Phytophthora* spp. and 19 strains of *Pythium* spp. were alive even after 5-years storage in the vapour phase. Production activity of sexual organs were confirmed in almost all of the revival cultures. The surviving strains after 1-month storage were found to tend to survive even after 5-years storage. The present method of cryopreservation was thought to be widely applicable for the genus *Phytophthora* and *Pythium*. In future, we will improve the present cryopreservation method for *Phytophthora* and *Pythium* to increase their viability after long term storage, and wil try its application to other genus of Oomycetes such as *Achlya, Aphanomyces, Dictyuchus, Thraustotheca, Plectospira, Saprolegnia*.

PS5-569-0709

A global strategy for the conservation of fungi

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Fungi are megadiverse, vital for ecosystem functioning and have numerous practical applications, yet their conservation lags a long way behind that of plants and animals. In fact, the majority of fungi have yet to be formally described, and biology and ecology are well understood for only a minority of named species. The recently re-formed IUCN Fungi Specialist Group (www.rbg.vic.gov.au/iucnsscfungi/), with members from all continents, is preparing a Global Action Plan for Fungal Conservation, to be published in 2008. A global approach will build on existing initiatives to conserve fungi and seek to provide better taxonomic, ecological and geographic coverage in fungal conservation awareness and planning.

Existing fungal conservation initiatives focus on getting public attention through inclusion of individual species (predominantly macrofungi) in national or regional RED lists. The ecological requirements of red-listed fungi, commonly combined with other red-listed organisms, are increasingly being used to analyze deficiencies of habitats and substrates and thereby to identify improved nature management. The Action Plan will provide standardized guidelines for the growing use internationally of IUCN threat categories to assess fungi for RED lists.

Both conservation and appropriate management of fungi and fungal habitats will be explored in the Action Plan. Decline of fungi must be detected early, to enable identification of causes and to suggest appropriate adjustments to ecosystem management. Hotspots, which are areas of high diversity or sites that contains threatened species, may be preserved as mycological reserves. Fungi restricted to rare or threatened hosts are themselves under threat, and more effort is needed to catalogue such fungi. For poorly known groups it is also vital to investigate the degree of congruence between fungal communities and the plant communities that commonly are the focus of conservation activities (and which may or may not provide an effective umbrella for conserving fungi). In situ conservation is the most desirable method, but ex situ conservation, such as through culture collections, should also be explored.

Efforts to conserve fungi must include initiatives to increase understanding of their diversity and ecological significance amongst educators, land managers, and those responsible for conservation policy. Iconic fungi (those that are beautiful, interesting or weird) are a means to raise the profile of all fungi, including the less conspicuous and more diverse microfungi. The complex interdependence of fungi with other organisms, such as in mycorrhizas, is also a useful means to engage conservation programs that currently focus almost exclusively on plants or animals.

PS5-570-0735

Keratinophilic fungi from Lonar meteorite crater soils (India).

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Lonar is the third largest meteorite crater in basaltic rock in the world, with a diameter of 1800 meter. It comes after Bosmatvi lake in Ghana, which has a diameter of 10000 meter and New Cubec in Canada with a diameter of 3500 meter. It has a history that goes back more than 50,000 years, when a meteor struck, near Lonar village in the Buldhana District of Maharashtra (19058'N, 76031'E) creating Lonar lake which has salty water in it.

Thirty two soils samples were collected from 6 sites in the vicinity of Lonar meteorite crater and screened for presence of keratinophilic fungi using hair baiting techniques for isolation. Seventeen isolates of keratinophilic fungi were recovered and identified by recognition of their cultures, macro- and micromorphological features. Their molecular characteristics was studied by sequences of ITS1-5.8S-ITS2 of rDNA region. Seven species of four genera were isolated viz. *Apanoascus durus,* (4.09 %), *Apanoascus punsolae* (24.59 %), *Auxarthron kuehnii* (1.63 %), *Chrysosporium indicum* (10.65 %), *C. tropicum* (18.85 %), *Chrysosporium* sp. (1.63 %), *Chrysosporium* state of *Ctenomyces serratus* (2.45 %).

PS5-571-0747

Cytotoxic assessment of waters harbouring watermoulds

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Water bodies are getting polluted unabated in India. The present study aimed to find out if the polluted waters that harbour watermoulds have any cytotoxic potentials.

The water samples were collected from the polluted sites and germinating roots of onion bulbs were treated with sampled water for 12, 24 and 48 hrs. Thereafter the roots were fixed in 1:3 acetic-alcohol and after 24 hrs stored in 70% alcohol. For cytological studies, these roots were hydrolyzed in 1N HCl and stained with haemotoxylin and squashed in 45% acetic acid. The number of dividing, non-dividing and abnormally dividing cells and the types of chromosomal abnormalities were scored from the temporary squashes. The data were adequately analyzed.

The effect of polluted waters was found treatment duration dependant. The mitotic indices decreased with the duration of treatments. The progressive increase in the frequency of interphasic cells with duration of treatment indicates that substances present in water act as mitotic inhibitors. Cytological examination of dividing cells showed that mitotic anomalies were also duration dependant. Various abnormalities include stickiness of chromosomes, lagging chromosomes, fragmentation, bridges, and multipolar ana-telophases. Stickiness of chromosomes was frequently observed followed by chromosomal breakage at metaphases and bridges at anaphases.

The induction of such chromosomal aberrations indicates that the polluted waters have cytotoxic potential. These waters harbour species of the fungal genera like *Achlya, Dictyuchus* and *Saprolegnia*. The occurrence of these fungi in such waters indicates their plasticity towards the environment.

PS5-572-0749

Ecology and conservation of grassland macrofungi

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Waxcap fungi (*Hygrocybe* spp.) are widespread and colourful components of nutrient-poor grasslands in Northern Europe, and are amongst the most highly visible representatives of the soil biota. Through surveys of the distribution of Hygrocybe spp. and of other macrofungal genera (eg Clavariaceae, Geoglossaceae) showing similar patterns of occurrence, a picture is emerging of the more important 'waxcap grassland' sites in the UK, and of those species in greatest need of protection. It is becoming clear that some sites in Northern and Western parts of the UK show exceptional diversity with >60 target species being present. Growing recognition of the international importance of some of these sites has recently led to their being given legal protection.
We have monitored the effect of various management regimes on fruiting of Hygrocybe spp. at a number of replicate grassland experimental sites. These include restoration experiments where it has been possible to monitor the recovery of grassland fungal following cessation of fertiliser and lime additions.
Fruitbodies of these fungi, subjected to stable isotope analysis were found to be highly enriched for 15N and depleted in 13C relative to soil and vegetation. These and other analyses have provided some insight into the nutritional biology of these fungi. Flow cytometric and microscopic analysis has also been used to elucidate patterns of spore germination and dormancy in a range of species.

PS5-574-0828

DIVERSITY OF MICROMYCETES IN LIBRARIAN ECOTOPES.

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Contamination of the book depositories by fungi and investigation of their regularities remain one of the complicated problem we face. Its vital importance has made it the subject of numerous investigations. The experiments were executed in four huge depositories of The National Library of Russia (Saint-Petersburg). Three of them have optimum thermohydrometric conditions for the preservation of documents but in fourth the conditions were significantly worse and there were found mouldy spots on books. In this reseach we used modern methods for recording the temperature, humidity, light, total concentration of spores and spesies identification of isolated fungi.
All investigated places notably differed. Data on 110 species were collected . We present the lists of isolated fungi for every investigated depository.The species composition was analyzed in detail. Direct measurement in different mycocomunities demonstrated that they are quantitatively and qualitatively diverse. .Mycological analysis throughout the year made it possible to study in detail the structure of micromycete community, to determine typical dominant (frequency, more than 50 %), typical common (frequency,30 to 50 %), typical rare (frequency, 10 to 30 %) and causal (frequency, less than 10 %) species. All these micromycets are potentially dangerous as a destroyers of paper and, furthermore, can induce allergic diseases. Ecological peculiarities of fungi were described carelessly. It was noted that libraries havs some typical species and we presented lists of isolated micromycetes. The overwhelming majority of *Penicillium* strains isolated from some environments were close to the species P. canescens Sopp and P. *natatum* Westling. Furthermore, the data obtained confirm, the earlier suggestions that the pathways of some particular species are similar and there is weaker effect of the outside microflora.
This study carries out a comparative analyses and provides a general discription of mycocomunities in library.

PS5-573-0830

The diversity and distribution of rust fungi of India

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India is rich in fungal diversity accounting for nearly 1/3 of the global diversity. Among several groups of Fungi the Rust Fungi (Uredinales-Urediniomycetes-Basidiomycota) are an important group of obligate plant pathogens causing . Rust fungi show a complex pleomorphic life cycle where a single organism produces different kinds of spore stages. Rust fungi show a very wide host range infecting a good majority of vascular plants viz., Pteridophytes, Gymnosperms, and Angiosperms (both Monocotyledonous and Dicotyledonous plants). Like their lifecycle the taxonomy of rust fungi is also complex.
So far nearly 630 species (both holomorphic and anamorphic taxa) distributed over 70 genera of rust fungi are reported from India. The members of angiospermic families viz., Poaceae, Cyperaceae of Monocotyledons and Asteraceae, Fabaceae of Dicotyledons provide host substrate to a majority of these taxa. Among the 70 genera the genus *Puccinia* with 340 species is the largest genus followed by *Uromyces* with 100 species. An account of various rust taxa, their distribution and other details will be presented.

PS5-575-0841

Comparisons between the rust flora of Tibetan East Himalaya and the rust floras of India, Nepal and Pakistan

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The rust flora of Tibetan East Himalaya is characterized by the predominance of eastern Asian species. One hundred twenty-nine species, amounting to 55.4% of the total known species in the region, are in common with the rust species of Japan.
Tibetan East Himalaya adjoins Indian subcontinent and geologically is situated at the suture line connecting Indian plate with Eurasian plate. The penetration of Indian-Malaysian floristic component to the Tibetan Himalaya is unavoidable. The southern slope of East Himalaya has a climate effected deeply by the monsoon from Indian Ocean and an abundant rainfall and a rich vegetation presenting sight of tropical rain forest are suitable for survival of rust fungi from India. Seventy-four rust species, amounting to 31.8% of the total known species in Tibetan East Himalaya, are in common with the rust species of India. It is worth mentioning that the primeval forest in the south to Medog adjacent to India is still inadequately explored. The Indian rust flora is exceedingly luxuriant, containing a number of phylogenetically primitive monotypic and small genera such as *Arthuria, Chrysocelis, Hiratsukamyces, Masseeëlla, Phragmidiella*, etc. which are still not known in Tibetan East Himalaya. The distribution of their host plants suggests that they are probably present in the tropical forest of the region and yet await the collectors.
Situated in Central Himalaya, Nepal is relatively similar to East Himalaya in forest vegetation. Comparison between the rust flora of Tibetan East Himalaya and that of Napalese Himalaya shows some similarities. There are 61 species in common, amounting to 26.2% of Tibetan East Himalayan total. The abundance of East Asian species suggests that the Nepalese rust flora is a continuation of the East Himalayan rust flora.
Pakistan is of generally typical climate of subtropical steppe and desert. The Himalayas, Karakoram Mts. and Pamir Plateau occupy the northern part of the country. The rust flora is quite different from that of the other parts of Himalaya. There are about 200 known species belonging to some 20 genera. Only 36 rust species in Tibetan East Himalaya, amounting to 15.5% of the total, are in common with the rusts of Pakistan. The tropical genera commonly found in East Himalaya such as Gerwasia, Hamaspora, Maravalia and Ravenelia are rare or not found in the vast areas of Pakistan inland. The connexion between the rust flora of Tibetan East Himalaya and that of Pakistan seems very weak.

PS5-576-0861

Metabolic and molecular biodiversity – evidence from a survey of New Caldedonian Fungi and New Zealand Xylariaceae

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Much work has been dedicated to fungal biodiversity inventories, using traditional morphology, and meanwhile even molecular approaches. The "innovative potential" of tropical fungi with respect to the discovery of novel secondary metabolites and other innovative products for application in the Biotech industry was advertised repeatedly. However, most of the lead compounds ever derived from fungi have been originally discovered from temperate species! There are only few exceptions, such as nodulisporic acid from a group of pantropically distributed endophytes [1]. We have attempted to correlate molecular and morphological data on species abundance vs. diversity of secondary metabolite production in fungi from tropical and temperate climates and wish to present results of two different projects.
a) A study on the xylariaceous genus *Hypoxylon* based on HPLC profiling [2] revealed an essentially redundant secondary metabolism in many of their species, independent from differences in molecular and morphological traits. In contrast, several apparently rare or endemic *Hypoxylon* spp. from New Zealand and the temperate Northern hemisphere were found to contain unprecedented metabolites. Most of the New Zealand fungi studied that produce specific metabolites were found from islands or other isolated areas.
b) During a survey of New Caledonian fungi from different habitats, carried out by a combination of molecular and chemical methodology (comparison of ITS nrDNA and dereplication by HPLC profiling), we found that about 35 % of the isolates contained several metabolites whose spectral data did not match with the entries of commercial secondary metabolite databases, and with a proprietary HPLC-MS library. The ITS nrDNA and HPLC data correlated very well; groups of strains with redundant DNA sequences generally showed similar HPLC profiles. It was occasionally even possible to predict metabolite production by the molecular data and chemotaxonomic information. Some strains from public collections whose sequences were retrieved from GenBank by BLAST searches showed similar HPLC profiles as the wild type isolates.
=> Attaining quality assurance by molecular phylogeny appears ideal to identify redundancies prior to screening, especially if and when microscopic/cultural characters do not allow for an effective morphological dereplication.
[1] J. Polishook et al. (2001) Mycologia 93: 1125–1137.
[2] V. Hellwig et al. (2005) Mycol Progr 4: 39–54.

PS5-577-0878

Mycology in a temperate riparian forest canopy – the fungal side of the LAK-Project

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Despite more than 20 years of intensive canopy research, mycology never played and important role in investigating the upper parts of a forest. Using the Leipzig Canopy Crane research facility (LAK) to gain access to the canopy of an inner-city floodplain forest, our research is the first of this kind to study fungal organisms that live in different niches within tree crowns.
Dead wood was collected between 10 and 33 metres above the ground to assess the diversity and ecology of decomposing 'canopy fungi'. Parallel to these studies the biodiversity of myxomycetes and allied organims (MMLO) was investigated on the same substrate (responsibility Prof. Dr. M. Schnittler, University Greifswald). Recently a third group was included in the project: Leaf-inhabiting endophytic fungi.
The mentioned topics are clearly biodiversity-orientated and concentrate on species richness, species turnover within the canopy (host and substrate preferences, vertical dispersal from shadow crowns to the upper layers), and ecology of the organisms.
Two spanning results are that the canopy of the investigation site can be viewed now as a convenient habitat for wood decaying fungi and MMLO, and that old trees with a large amount of dead branches in their living crowns, in particular the species *Quercus robur*, are crucial for the maintenance of fungal biodiversity in such forest ecosystems.

PS5-578-0920

Queensland DPI&F Biosecurity – enhancing North Queensland's protection against exotic plant diseases

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The Queensland Department of Primary Industries and Fisheries (DPI&F) Biosecurity group undertakes surveillance and response programs for exotic plant pests and diseases in far north Queensland (FNQ), Australia. FNQ has an increased risk of exotic pest and disease incursions due to its proximity to south-east Asia and Papua New Guinea, where many of the threats are present. Early detection of incursions provides an opportunity to control or eradicate them, and lowers the potential impact on Queensland's primary industries. In collaboration with the Australian Quarantine and Inspection Service (AQIS) and the Northern Australian Quarantine Strategy (NAQS), early warning and response surveillance is undertaken on the Cape York Peninsula, Torres Strait Islands and urban centres south to Mackay. A target list is used to focus surveillance efforts. The target fungal plant diseases include black Sigatoka and Panama disease (tropical Race 4) of banana, powdery mildew and scab disease of citrus, guava rust, grapevine leaf rust, downy mildew of maize, sorghum and sugarcane, potato late blight (A2 strain) and sugarcane smut.

PS5-579-0984

The rusts of Myrtaceae

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The nomenclature of the species of rust fungi occurring on species of Myrtaceae is reviewed. The Myrtaceae is a large monophyletic family comprising 2 subfamilies, 17 tribes, about 130 genera and about 4600 species.
Three teleomorph and five anamorph species of rusts are accepted: one species of *Phakopsora*, two species of *Physopella*, two species of *Puccinia* and three species of *Uredo*. Each of these taxa can be recognised using only morphological characters.
The known hosts of each of the myrtaceous rusts are recorded. With the exception of *Puccinia psidii* and two species of *Uredo* each of the rusts is known only from a single host genus. There is no indication there has been a radiation of rust taxa on Myrtaceae rather it seems speciation has followed rare host jump events.
The widespread Central and South American species *Puccinia psidii*, guava rust, and its uredinial anamorph, is now known to occur on species in both subfamilies of Myrtaceae including one of two tribes of the subfamily Psiloxyloideae and 7 of the 15 tribes of subfamily Myrtoideae, a total of 20 genera and 71 species. Susceptibility to *Puccinia psidii* seems to be low amongst species of Myrtaceae from the Americas but much more common amongst taxa from Asia, Australia and the Pacific.
Puccinia psidii is now established in Hawaii and represents a significant biosecurity risk to Australasia, the Pacific and east Asia where species of Myrtaceae are often a dominant component of the flora and a major determinant of biodiversity.

PS5-580-0989

Land use systems and distribution of *Trichoderma* species in Embu Region, Kenya

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The distribution of *Trichoderma* spp in soils of Embu region in relation to land use practices was investigated. The study area was chosen because of its significant land use intensification. Soil washing and dilution plate techniques were used to recover *Trichoderma* spp from the soil samples. The fungal isolates were identified and assigned to eight species. Greater populations as well as a wider range of species were obtained in soils collected from the natural forests while coffee farms were the poorest ones. Land use affected the distribution of *Trichoderma*. Napier farms had the highest abundance of this fungus. The species which showed the highest incidence in all cases was *T. harzianum*. Plant type was a major determinant of the occurrence of this fungus. *Trichoderma* favored plants with shallow and widespread rooting systems, to the deeply rooted perennial coffee and tea trees. The age of the plants also was a driving factor. Both inorganic and organic fertilizers are used in the region. There was a negative correlation between amount of chemical fertilizers and abundance of the fungus. Organic fertilizers were used exclusively in napier farms that had the highest fungal abundance. Soil pH and amount of phosphorus were limiting and influenced the occurrence and abundance of this fungus. However carbon and nitrogen were not limiting though they were high in the forests and napier farms where the fungus was also abundant. *Trichoderma* showed tolerance to soil acidity since it was abundant in the most acidic soils under napier. Land intensification affected *Trichoderma* distribution negatively.

POSTER SESSION 7 INDUSTRIAL MYCOLOGY

PS7-581-0044

Studies on the production of the bioactive secondary metabolite, taxol - an anticancer drug by Phyllosticta spp.

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Taxol, an important and expensive diterpenoid anticancer drug, which is especially targeted to treat breast and ovarian cancers, was originally isolated from the bark of Pacific Yew, *Taxus brevifolia*. Taxol is synthesized by Yew species is found in extremely low amount. However, a full course of treatment for a patient may require 2 g of taxol, administered several times over many months. This amount of taxol would require the felling of 12 or more, large Pacific Yews of 100 years old and cost over US$ 10,000 for the drug alone. Presently, all taxol in the world's market has originated from *Taxus* spp. The supply issue is further complicated by the scarcity of the Yew tree. Although chemical synthesis of taxol has been achieved, the process is too expensive for commercialization. Ultimately, in order to lower the price of taxol and make it more available, a fermentation process involving micro-organisms would be the most desirable and alternate source of supply. In the present study, 12 different species of *Phyllosticta* (both pathogenic and endphytic species), a coelomycetous fungi isolated from South India were screened for the production of taxol in MID and PDB medium. The putative fungal taxol along with authentic taxol were analysed by different analytical methods viz. Thin Layer Chromatography, UV and IR spectroscopy and High Performance Liquid Chromatography. Of the 12 species tested, P. *tabernaemontanae* produced high content of taxol followed by P. *melochiea*, P. *spinarum,* P. *dioscorea* and P. *citricarpa*. The results and significance of the findings are discussed in detail.

PS7-582-0045

Effect of photoperiod on the growth and taxol production of *Colletotrichum capsici*

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Anthracnose fungus, *Colletotrichum capsici* causing fruit rot in Chill is one of the major worst and baffling disease in India. The growth of the fungus in invitro is considered to be very important in the study of disease development and control aspects of plant pathology. Present study was undertaken to find out the comparative effect of different photoperiods (light, dark and light/dark) on the growth (Radial growth, biomass, total proteins, carbohydrated and lipids) of *C. capsici* on different medium.

Nowadays, unexploited microbial biodiversity have been applied in the field of new drug discovery. Taxol, an important and expensive diterpenoid anticancer drug, which is especially targeted to treat breast and ovarian cancers, was originally isolated from the bark of Pacific Yew, *Taxus brevifolia*. From the plant source the amount of taxol production is found to be very low and the cost of the commercially available taxol drug is highly expensive. Ultimately, in order to lower the price of taxol and make it more available, a fermentation process involving micro-organisms would be the most desirable and alternate source of supply. Apart from pathogenic nature, the fungus also serves as an excellent source for the production of novel bioactive compounds. In the present study, productions of taxol by the fungus in different photoperiod were also carried out. The results and significance of the findings are discussed in detail.

PS7-583-0084

Differential breakdown of pesticide mixtures by *Trametes versicolor* and *Phanerochaete chrysosporium* by production of hydrolytic enzymes under different soil water potentials in soil microcosms

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Bioremedial strategies require fungi to be able to breakdown mixtures of pesticides effectively over a range of environmental conditions in soil. This study has addressed this problem under realistic soil conditions and measured the relationship between production of the necessary enzymes and rates of degradation of pesticide mixtures in soil microcosms under different soil water potentials over periods of 3 months.
Soil microcosms modified to -0.7 and -2.8 MPa water potential contaminated with a mixture of pesticides (simazine, trifluralin and dieldrin, 10 mg kg-1 soil) were inoculated with *T.versicolor* and *P.chrysosporium* grown on wood chips and temporal studies used to examine degradation rates at 15ºC for up to 12 weeks. Extracellular enzyme (cellulose, dehydrgenase, and laccase) production was quantified in soil. Respiration was also used to compare the effect of different treatments.
The two test isolates successfully grew and produced extracellular enzymes in soil at both water potentials. Respiratory activity was enhanced in soil inoculated with the test isolates, and was generally higher in the presence of the pesticide mixture, which suggested increased mineralization. Cellulase and dehydrogenase was also higher in inoculated soil than in the control especially after 84 days incubation. Laccase was produced at very high levels, only when *T.versicolor* was present. Degradation of the three pesticides by *T.versicolor*, after 6 weeks, was enhanced by 46, 57 and 51% respectively for simazine, trifluralin and dieldrin over that in natural soil. *P.chrysosporium* also significantly enhanced degradation rates over controls, especially in the wetter treatment. There was a correlation between some enzyme production in soil, degradation rates and other indicator criteria. This
This study shows that even under quite dry conditions (twice the wilting point of plants) fungal bioremediation of mixtures of pesticides occurs mediated by a range of enzyme activities

PS7-584-0090

Medium optimization for the production of the secondary metabolite squalestatin S1 by a *Phoma* sp combining orthogonal design and response surface methodology

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There has been interest in finding statistical means for optimising the production of high value products by economising on the number of experiments that need to be practically carried out. This study was carried out to address this issue by using a *Phoma* species which produces the cholesterol-lowering suite of polyketides (squalestatins).
In the present work, a combined statistical approach of orthogonal design (L27(313), response surface techniques and polynomial regression were applied to optimize the composition and concentration of a liquid fermentation medium for the production of squalestatin S1 by the *Phoma* species. Optimal conditions for maximal titres and productivity were determined based on thirteen parameters at three different levels. Initially, a screening design methodology was used to evaluate the process variables which were relevant to S1 titre and the response surfaces applied to find optimal regions for production.
The sources of carbon and concentration, and their interactions with oily precursors were statistically significant factors. The combined orthogonal design and response surface methodology predicted optimal conditions of 273 mg l-1 of squalestatin S1. Confirmatory experiments of the optimal medium composition produced titres of 434 mg l-1 in a five day fermentation at 25oC. This represented a 60% improvement in the maximum titre predicted, and a two-fold higher productivity when compared with reported S1 yields of various fungal species. Surface response curves were produced to show the impact of different individual and combined factors and their impacts on S1 production.
This combined statistical approach enables rapid identification and integration of key medium parameters for optimising secondary metabolite production and could be very useful in pharmaceutical screening programmes and also in optimising the production of heterologous proteins.

PS7-585-0097

Production of the biocontrol agent *Phlebiopsis gigantea* in controlled environmental and nutritional media for control of wood decay

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Heterobasidion annosum, a tree pathogen, is ubiquitous in the environment and causes financial losses for the forestry industry. It has been found that the saprophyte, *Phlebiopsis gigantea*, can reduce losses from *H. annosum*, by out-competing for woody resources. The natural population of spores of *P. gigantea* can be augmented by the application of a spore suspension, to the stumps, at the time of tree-felling.
Environmental studies have been carried out to assess the fitness of different isolates of the antagonist relative to the pathogen. An index od dominance was established under different environmental regimes. The potential use of the antagonist is dependent on the ability to produce a high quality inoculum with ecological competence. Studies were conducted in liquid culture, immobilised gels, and solid substrate to evaluate the production of spores. Subsequently, fluidised bed drying was used for the first time to make formulations of this biocontrol agent. The effect on viability under different environmental conditions was assessed.
Competitiveness was affected by environmental factors, especially water availability. These indicate that the antagonist was effective over a narrow environmental niche which needed to be maintained for efficacy in the field. Under different osmotic and matric conditions the efficacy of strains of the antagonist was more sensitive than the pathogen, especially under drier conditions.
Studies have been carried out to examine potential for liquid, immobilised alginate beads and solid substrate fermentation systems for optimising production of *P.gigantea*. Liquid culture studies were variable regardless of available nutrients and ecophysiological stresses imposed. However, temporal studies on immobilised beads and solid substrate based on *Pinus sylvestris* sawdust gave >Log10 7 viable oidia g-1 in the best treatments. Scale up, preservation studies and analyses of the endogenous reserves of the best treatments are in progress to identify specific quality characteristics.
These studies have shown the best systems to use for the production of this biocontrol agent. The importance of speficying the range of conditions over which effective establishment and control can be achieved is important. The production of high quality spores which have retained viability and shelf life can be achieved for this biocontrol agent based on the knowledge gained in this work.

PS7-586-0102

Selection of Ligninolytic Fungi and Application to Bioremediation of Contaminated Water and Soil

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Pollution with persistant organics has become major ecological problem. Ligninolytic fungi (LF), microorganisms of choice for bioremediations, are able to efficiently degrade a great number of pollutants using oxidative enzymes with relatively low specificity. The work focused on selection of powerful LF degraders, development of remediation techniques, and estimation of degradation efficiency with various synthetic dyes.
Screening of LF strains with high biodegradation capacity was carried out using polymeric and non-polymeric dyes. LF column reactors consisting of 3-6 g dry solid material were used for removal of dyes at 0.15-10 g.l-1 in culture media and textile industry effluents [1]. Decolorization of dyes was measured colorimetrically and degradation products characterized by HPLC-MS. 100-ml Erlenmeyer flasks contain-ing straw-grown LF inoculum were used for bioremediation of anthraqui-none-dye-contaminated soil by explorative fungal mycelium [2]. The re-moval of pollutants was detected colorimetrically after extraction.
Selected fungus Irpex lacteus decolorized 94-100% of azo-, anthraquinone-, triphenyl methane-, phthalocyanine- and thiazine dyes (150 mg.l-1) within 1 week, degradation rates were in the range of 105-213 Ìg dye.h-1 at a flow rate of 1 ml.h –1, 22 °C and a forced aeration. Products of degradation of the azo dye Reactive Orange 16 were determined by HPLC-MS. Decolorization rates in diluted, pH-adjusted textile industry effluents containing Acid Black, Cassulfon Blau, Drimaren Red, Drimaren Blue, Eybl Green, Eybl Red or Remazol Green were 95, 94, 90, 83, 81, 30 and 25% in 2-7 d, respectively. Besides column reac-tors, rotating disc reactors could also be used for decolorization of dyes.
LF in the form of immobilized cultures and explorative mycelium could be used for efficient removal of various types of synthe-tic dyes from water and soil and for remediation of textile coloring liquids.
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PS7-587-0109

A qualitative investigation on tea garden air fungal pollution in the north of Iran, Gilan Province Estern Region

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Fungi Are Obiquitous And Infact Account For At Least 25% Of The Earth's Biomass So Abundant In Many Area Or Spaces Serving Optimum Temprature And Humidity .Fungi Have Long Been Know To Affect Human Life Status In Various Efforts Including Soil Habitation, Plant / Animal Parasitism And Food / Drink Decaying, With Possible Concomitant Production Of Mycotoxins, Tissue Infections And Immune Stimulation Or Combat .Ecologic Microbiota Of Fungi Can Grow In Initial Area Even The Region May Play More Improtant Role Than Climatic Forces Such As Seasonal Fluctuations On Fungal Flora .It Must Be Pointed Out That Outdoor Fungi Concentrations Are In Relation To Indoor Counts But In Different Genera So The Taxa Identified Is Much More Important Than The Absolute Number Of Colony – Forming Units .Occupational And Environmental Health Professionals Are Confronted With Issues Concerning Bioaerosol Harmness Regarding Investigation Both Indoor And Outdoor Fungi In Complaint And NonComplaint Area .In This Respect Totally 62 Regional Typical Tea Gardens Dust Bioaerosols Were Sampelified And 1005 Mold Colonies Were Isolated Due To Driect Microscopy And Culture – Based Inspective Conventional Mycologic Methods , During May To August 2005. Finaly 26 Different Genera Of Habitate Fungi Were Collected And Confirmed From 194 Conducted Plates. Of Defined Geographic Location , This Is The Largest Study Of Air Borne Outdoor Fungal Species With Rutine Protocol To Date . Related To Nouniformity Of Any Specific Guide Lines Measuring Outdoor Environmental Fungi And Determine Potentially Outcoming Deseases Due To Exposure Making Interpretation Of Existing Data Difficult , Air Testing Methodology Must Be Expanded And Evaluated .To Build A Model To Clarify Determinants Of Airborne Fungal Populations And Health Assessment In Public Area Within A Defined Geographic Location And Labor, Additional Studies Are Needed To Document Any Suspected Relations Prospectivly As Well As Retrospectivly.

PS7-588-0114

Ligninolytic enzyme production in *Pleurotus ostreatus* depending on the medium composition and cultivation conditions

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Pleurotus ostreatus is an edible and medicinal species that belongs to the group of white rot fungi due to its ability to produce extracellular ligninolytic enzymes (laccase, Mn dependent peroxidase, versatile peroxidase, and aryl-alcohol oxidase), and modify and degrade lignin. P. ostreatus is commercially cultivated on different lignocellulosic materials such as sawdust, paper products, and most agricultural wastes that are produced in enormous amounts worldwide. The purpose of this investigation was to study the effect of different carbon and nitrogen sources, and raw plant materials under conditions of submerged fermentation and solid-state fermentation, on the laccase and peroxidases production in *P. ostreatus*.

The influence of 7 inorganic (carboxymethyl-cellulose sodium salt, cellulose, glucose, maltose, D-mannitol, D-gluconic acid sodium salt, and xylan) and two organic carbon sources (grapevine sawdust and mandarine peels) under submerged and solid-state fermentation conditions was studied. Effect of 5 inorganic (ammonium chloride, ammonium nitrate, ammonium phosphate monobasic, ammonium sulfate, and potassium nitrate) and three organic (bacteriological peptone, casein acid hydrolysate vitamin free, and corn step liquor) nitrogen sources was studied by their addition to the medium with optimal carbon source under optimal cultivation conditions. Enzymes activities were determined spectrophotometrically, by syringaldazine for laccase and phenol red for peroxidases.

Laccase was produced under both studied conditions using all of the investigated carbon and nitrogen sources, while significant peroxidases production occurred only in solid-state fermentation. The highest levels of laccase, Mn depended peroxidase and versatile peroxidase activities were found in solid-state fermentation of grapevine sawdust (378,24 U/l; 4,77 U/l; 6,33 U/l).

After purification of extracellular crude enzyme mixture of *P. ostreatus* which was grown under solid-state fermentation conditions with grapevine sawdust, three laccase peaks (14 U/l, 124 U/l, 873 U/l) and two peaks of activity against phenol red in presence and absence of external Mn2+, respectively (12.6 U/l and 4.4 U/l) were revealed.

In the medium with the best carbon source for enzymes production (grapevine sawdust) peptone and ammonium chloride were the optimal nitrogen sources for laccase synthesis (466.2 U/l and 501 U/l). The peak of activity against phenol red in presence of external Mn2+ was found in the medium with peptone and ammonium nitrate (59,1 U/l and 53,5 U/l), while the peak of activity in absence of external Mn2+ was with casein acid hydrolysate vitamin free and potassium nitrate (17,0 U/l and 16,3 U/l).

PS7-590-0118

Isolation and *in vitro* cultivation of *Auriculoscypha anacardiicola*

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Auriculoscypha (Basidiomycota, Septobasidiales) is a remarkable genus that seems to be endemic to south-west India. It forms part of an interesting fungus-insect-plant co-existence and interaction. It seems to be restricted to the bark of a few, mostly anacardiaceous, trees and is invariably associated with a coccid *Neogreenia zeylanica*. It is a monotypic genus and *A. anacardiicola* is the only known species. Isolation of pure cultures and their in vitro cultivation are fundamental steps in the understanding of the biology of any fungus and are all the more important in the case of an obligate insect-symbiont like *Auriculoscypha*. For this reason we decided to attempt isolation and in vitro cultivation of *Auriculoscypha anacardiicola*. This project had three aims to achieve: 1) to isolate a pure culture of A. anacardiicola on a solid culture medium; 2) to trace the step-by-step development of a mycelium of A. anacardiicola from a germinating basidiospore; 3) to compare the growth-rate and colony characters of cultures of A. anacardiicola on six fungal culture media. Conventional mycological methods to isolate and cultivate fungi were employed. Isolation of *A. anacardiicola* proved to be a difficult task but in spite of this a dependable method for the isolation and in vitro cultivation was accomplished in this study. Allowing basidiospores from fruit bodies to fall directly on tap water agar was the only way by which *A. anacardiicola* could be isolated in culture. The basidiospores germinated in any of the following three ways: by germ tube formation; by formation of secondary ballistospores; by formation of secondary blastospores. The mycelium of *A. anacardiicola* develops, at least under in vitro conditions, only from a yeast phase. This yeast phase then passes through a brief pseudomycelial phase before becoming normal mycelium. Colonies growing on Malt-Yeast-Peptone agar showed the largest overall mean colony diameter and this medium is considered best for A. anacardiicola. Czapek-Dox agar (CDA) is not supportive of growth of A. anacardiicola. Of all the six media tested, CDA is distinct in that it is the only medium containing only sucrose as the carbon source. It is quite possible that A. anacardiicola lacks enzymes needed for sucrose utilization.

PS7-591-0140

The use of denaturing gradient gel electrophoresis (DGGE) to identify key fungi involved in the commercial mushroom composting process

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Good compost for growing the button mushroom Agaricus bisporus is the key limiting factor to highly profitable production. Compost failure can drastically reduce yields and economy of production and has forced farm closures. The key to high yielding compost, and thus economic yields, is the amount and types of carbohydrates and minerals present in the finished compost. These components are produced by the controlled development of micro-organisms that sequentially break down the straw and nitrogenous material. It is this controlled development of the microbes and their breakdown products that provides the potential for final mushrooms yields.
In this study we used DGGE with fungal specific primers targeting the 18S ribosomal DNA region to profile the fungal population of both phase I and phase II composts. Advantages of using this technique are that it is includes non-culturable and slower growing fungi, it gives a good indication of the diversity of fungal species present, and the level at which diversity is detected (i.e. strains vs species vs genus) can be altered with the primers used. Six known important compost fungi were developed as standards for the system. Results from preliminary studies revealed issues with sampling and PCR replications to yield consistent and reliable results. A number of trials were subsequently conducted to optimise the process and provide more reliable results. Bands of interest were excised from gels, sequenced and identified using a Genbank blast search. A number of fungi were identified that had not been previously described from mushroom compost. This DGGE method will now be employed to compare composts from different farms using different composting processes, as well as, comparing 'good yielding' compost with 'poor yielding' compost to identify key indicator fungi responsible for each of the outcomes. Information gained will be used to develop tools to monitor key indicator fungi throughout the composting process to predict when production modifications may be necessary to ensure consistently good yielding compost is produced.

PS7-592-0152

Bioactivities of *Phellinus linteus* Extracts Growing in Chinese Herbal Formula Substrates

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Phellinus linteus, a well-known orange color mushroom growing on mulberry tree, is scattering around the oriental countries such as Korea, Japan, and China. In Korea and Japan, the fungus of the genus *Phellinus* in the family of Hymenochaetaceae has been used as a traditional herb medicine for years in oriental countries, especially common use in cancer treatment. Mycelia of the fungus were grown well when cultured on ten different solid media. The mycelia growing on different solid media are yellowish in color. The preliminary results indicate that the capability of inhibition of tumor cells including fibrosarcoma cell (HT 1080), human breast cancer cell (MCF 7), human cervical cell (HeLa), and human colon adenocarcinoma cell (COLO 205) treated with the hot water extracts from SBB, BN, and LW media is significantly potential in healthy food. In antibiotic activity, 10mg/mL of P. linteus hot water extract inhibits dominantly sportulation of *Penicillium citrinum*. The thin layer chromatography and high pressure liquid chromatography showed that the culture of *P. linteus* had produced new compounds derived from BN substrate. Meanwhile, hot water extract of *P. linteus* growing in YM substrate obviously proved the anti-inflammatory activity according to the assay of inhibiting NO activity of mouse macrophage cell (RAW 264.7) stimulated by lipopolysaccharide (LPS 2 ?g/mL).

PS7-593-0196

An experimental strategy towards directing biosynthesis of communesin alkaloids by a *Penicillium* sp. in submerged fermentation

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Communesins were discovered as metabolites of several *Penicillium* isolates in the 1990s, and their complex structures shown to have indole and isoprene components. Various biological activities of potential pharmaceutical and agrochemical interest were also recognised. The compounds are now regarded as rather typical of P. expansum and some related species (Samson & Frisvad, 2004).
Industrial development for potential product scale-up involved not only optimised pilot-scale fermentations, but also more fundamental aspects of biosynthesis and developing potential for generating structural analogues through directed biosynthesis. Experiments with 14C-labelled precursors showed biosynthetic involvement of tryptophan, acetate, mevalonate and methionine. 14C tryptamine, generated from 14C-tryptophan by phenylalanine decarboxylase from *Streptomyces faecalis*, was also incorporated efficiently into communesins. Some radiolabelled tryptophan analogues were synthesised (e.g. 14C-6-fluorotryptophan from 6-fluoroindole and 14C-serine utilising the hyper-expressed tryptophan synthetase of an *E.coli*) and revealed rather high enzyme specificity in communesin biosynthesis. However, 5-bromotryptophan and 6-fluorotryptophan could be accepted, but the latter only into a mono-fluorinated analogue as shown by mass spectrometry. This indirectly indicated that in the di-indole moiety of communesins the indolic precursor that does not become N-methylated is the only one for which a precursor indolic analogue can substitute. Consequently, an experimental mutagenic strategy was developed to select a variant lacking tryptophan decarboxylase, which might more readily accept a tryptophan analogue. After NTG treatment of spores giving 99% kill, 1500 survivors were each grown in 100µl medium in 96-well microtitre plates with carboxy-14C-tryptophan and covered by filter paper impregnated with barium hydroxide. Autoradiography revealed 4 colonies with low activity in decarboxylating tryptophan, with altered phenotypic characters, and with suppressed communesin biosynthesis though reversible by added tryptamine. Prospects will be discussed.

PS7-594-0225

Evaluation on the utilization possibility of waste mushroom logs as biomass resource using enzyme analysis

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Environmentally-friendly biomass has been special attention as a new material of alternative energy. A lot of waste mushroom logs after production of oak mushroom are suitable lignocellulosic material for the production of bioethanol because the degradation of lignin was caused by the lignin degrading enzymes of Lentinus edodes. In order to investigate the ability of waste mushroom logs as biomass, characterization of enzymes of L. edodes related to cellulose degradation were examined.
The measurement of crystallinity in normal woods and mushroom logs during cultivation period was carried out by powder High Resolution X-ray Diffractometry. Chemical component analysis of wood powder was measured by TAPPI test method. Cellulase activity was determined by endo-1, 4-beta-glucanas, cellobiohydrolase, beta-glucosidas and xylanase. Protein was determined by Bradford with bovine serum albumin. Enzyme was purified by FPLC using ion exchange and size exclusion chromatography equilibrated with 20mM sodium acetate buffer, and identified by LC/MS-MS. Activity of purified enzyme was determined in different pH(6~8) buffer solution and temperature(30~70).
The crystallinity of waste mushroom logs which after the inoculation was drastically decreased during the cultivation. On the cultivation of fungi, lignin contents of normal wood and waste mushroom logs were decreased, however, holocellulose contents were increased relatively. The cellulases activity of the waste mushroom logs was higher than that of normal woods. Especially, the activity of xylanase which degraded hemicelluloses was three times higher than that of normal woods. When the waste mushroom logs were used as carbon source, a new protein band was appeared at 35kDa. According to the results of purification and identification of this band by LC/MS-MS, it was very homologous to the xylanase from Aspergillus terreus and the highest Xcorr of 1.737 was determined at amino acid of RKWI SQGIPIDGIG SQTHLGSGGS WTVKD originated from A. terreus.
Waste mushroom logs were renewable resources because it had low crystallinity and low lignin contents. Especially, it had potential of degradation of hemicelluloses by secreted xylanase from L. edodes, Based on these results, waste mushroom logs had enough potential as a material for developing alternative energy.

PS7-595-0226

Study on using A. niger XP isolated from soy bean waste to produce acidic phytase for animal using cassava bagasse waste.

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Phytase has an important role in current farming systems since they solve both the nutritional and environmental problems. At present, all phytases for farming in Vietnam are imported. In order to reduce the import of enzyme , this work has been done for obtaining cheap phytase of A. niger XP by SSF using cassava bagasse waste that could reach millions of tones per year but caused serious environmental pollution in Vietnam.

The enzyme activity of the phytase was determined by the method of Shimizu, M.et al.,1992. Both conventional and D1, D2 sequencing method were used for identification of the mould producing phytase. TLC and ELISA methods were applied for detection of Ochratoxin A production of this strain. In vitro digestion of the feed by *A. niger* xp was performed according to the method of T.Matsui et al.,2000.

From soybean waste, a strain of *Aspergillus* which could produce high acidic phytase with two optimum pH (one of 2.5 and the other of 5.5) and optimum temperature of 55oC was isolated. The *A. niger* xp phytase could effectively release Pi from many substrates used for feed in Vietnam such as corn, soybean, rice bran, commercial feed HI – GRO and C20. The enzyme is notable for its stability in the acidic condition and action of pepsin. When 20 unit of enzyme was mixed with 10g feed with an addition of 20000 IU pepsin/g at pH=2.0 and incubated for 2h in vitro, its activity was remained intact and it could release about 1300 ?mol Pi /g from rice bran. Therefore suggestion that, this enzyme could be active when introduced to feed digestion in stomach of animals. This strain was identified to be *A. niger* Tiegh, hereafter it was named as *Aspergillus niger XP*.

Aspergillus niger XP was subjected to SSF using cassava bagasse waste for producing of phytase. The effect of fermentation condition was investigated and the result obtained showing that It could produce 4.8IU/g ferment mashes when subjected to SSF using cassava bagasse waste moisten to 65% with minerals solution (KNO3 3.0 ; K2HPO4 : 1.0g; MgSO4 0.5 ;KCl: 0.5 FeSO4 : 0.01;H20 :1000 ml) at 34oC, pH = 4,0 for 40h. The ferment mash was determined to be free of Ochratoxin A.

All obtained data show that it is high possible to employ A. niger xp for converting cassava bagasee waste to obtain low cost phytase using for enzyme feed in Vietnam.

PS7-596-0244

Isolation and characterization of a anticholesterolemic ,ß-hydroxy-ß-,methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor from Pholiota adiposa

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For the purpose of development of a new anticholesterolemic drug or nutraceuticals from natural sources, screening of a potent HMG-CoA reductase inhibitor-producing mushroom and optimal extraction condition of the HMG-CoA reductase inhibitor were investigated. The methanol extracts of Pholiota adiposa ASI 24018 showed the highest HMG-CoA reudctase inhibitory activity of 55.8%. The HMG-CoA reductase inhibitor of Pholiota adiposa ASI 24018 was maximally extracted when it was treated with methanol at 30? for 12hrs. After purified the HMG-CoA reductase inhibitor by systematic solvent extraction, gel column chromatography and RP-HPLC, finally obtained the HMG-CoA reductase inhibitor with an activity of IC50 6.8ug. Molecular weight of the purified HMG-CoA reductase inhibitor was 765.4Da by LC-Mass, NMR and FT-IR spectrophotometry. The purified HMG-CoA reductase inhibitor were soluble in hexane, chloroform, methanol and DMSO, wherease it was water-insoluble. It also had a maximum absorption spectrum at 274nm. [This study was supported by ARPC(Agriculture R&D Promotion Center) technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea]

PS7-597-0245

Isolation and charaterization of a novel antithrombotic compound from mushrooms.

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The isolation and characterization of a platelet aggregation inhibitor and fibrinolytic compound were performed on various extracts from mushrooms for development of a novel antithrombotic compound. The highest platelet aggregation inhibitory activity was 81.2% in the ethanol extract from fruiting body of *Inonotus obliquus* ASI 74006 and also were high in the ethanol extract from fruiting bodies of *Fomitella fraxinea*. The ethanol extract from the mycelia of *Agaricus blazei* Murill. ASI 1174 showed the strongest fibrinolytic activity as 9.6 unit. However, fibrinolytic activities of other mushrooms were low or negligible. Therefore, we finally selected Inonotus obliquus ASI 74006 as producer of a potent antithrombotic compound. The maximum platelet aggregation inhibitory activity was found when the mycelia of Inonotus obliquus ASI 74006 was extracted with ethanol at 80? for 12 h. The platelet aggregation inhibitor was purified by systematic solvent fractionation, ultrafiltration, Sephadex G-10 column chromatography, and reverse-phase HPLC. The purified platelet aggregation inhibitor is a novel tripeptide with a molecular mass of 365 Da, having a sequence of Trp-Gly-Cys. The purified platelet aggregation inhibitor also showed high platelet aggregation inhibitory activity in Institute of Cancer Research (ICR) mice. [This study was supported by ARPC(Agriculture R&D Promotion Center) technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea]

PS7-598-0249

Distribution Profiles of Ginsenosides in Korean Ginseng(Panax ginseng C. A. Meyer) Cultured with *Ganoderma lucidum* Mycelium

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This research was conducted to compare the biotransformation of both ginsenoside derivatives and ,-glucosidase activity in korean ginseng cultured with *Ganoderma lucidum*. Solid fermentation was performed to prepare the ginseng media by inoculating the sterilized ginseng radix with Ganoderma lucidum mycelium and cultured at 25? for 30 days. Results revealed that culture caused a marked change in the compositions of ginsenosides and a significant reduction in the content of ginsenoside Rb1, compared with the uncultured sterilized ginseng radix. However, the content of ginsenoside Rd increased as culture age increased. The extent of decreased ginsenoside Rb1 and increased ginsenoside Rd contents varied with *Ganoderma lucidum* used. Among the various cultured ginseng radix prepared, *Ganoderma lucidum* KFRI 011 biotransformed from Rb1 to Rd and showed the highest level of Rd. The percentages of Rd in total ginsenoside increased from an initial ?6.6% to 28.3% after culture by *Ganoderma lucidum* KFRI 0110. In comparison, the percentages of Rd in total ginsenosides found in ginseng radix inoculated with Ganoderma lucidum KFRI 0201 ranged from 6.6% to 17.5% after culture. Compound K as biotransformed ginsenoside was also identified with FAB-MS, 13C-NMR, 1H-NMR. In addition, the increases of the bioconverted ginsenoside content and ,-glucosidase activity during the culture age showed a similar trend.

PS7-599-0317

Isolation, Selection, and Optimization for Xylanase Production from *Aspergillus niger* from Soil in Thailand

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Xylanase producing fungi were isolated from soil in Thailand. Thirty strains of fungi different in macroscopic morphology were isolated. Among these 30 strains, no. 27 was selected as potential xylan-digesting fungi. The xylanase activity of the fungal strain no. 27 was determined comparing with a reference strain, *Aspergillus foetidus* TISTR 3159 by measuring the ratio of the clear zone to that of the colony on the agar plate and determining xylanase activity in liquid cultivation. At pH 6, fungi no.27 produced xylanase with the highest ratio of the clear zone to that of the colony of 3.57 and the enzyme activity of 33.82 U/ml. The optimum cultural conditions for xylanase production by fungi no. 27 were studied. The maximum xylanase production was obtained in a medium containing 30 g/l corn cobs as carbon source, 0.3 g/l urea, 0.25 g/l proteose peptone, 0.05 g/l yeast extract and 0.3 g of (N)/l (1.4 g/l) of (NH4)2HPO4 as nitrogen sources , 0.2 g/l KH2PO4, 0.3 g/l CaCl2.2H2O, 0.3 g/l MgSO4.7H2O and 2 ml/l Tween 80 at the initial pH of 6.0, a rotation speed of 200 rpm, incubation period of 6 days, and the temperature of 30∞C. In the presence of selective medium, the maximal xylanase activity was seven times higher than that in standard medium. The maximum xylanase and cellulase activity of fungi no. 27 were detected after 5 days of cultivation at 236.53 U/ml and 0.22 U/ml, respectively. The maximum xylanase and cellulase of reference stain *A. foetidus* TISTR 3159 were obtained on the 5th day of incubation at 211.90 and 0.28 U/ml respectively. The fungi no.27 was identified as *Aspergillus niger*.

PS7-600-0319

Isolation, Selection, and Optimization for Xylanase Production from Aspergillus niger Isolated from Soil in Thailand

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No abstract available.

PS7-601-0327

New cropping system to improve productivity of Gastrodia elata

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Gastritroda elata is perennial plant without chlorophyll only absorbed nutrition by rhizomorph of *Armillariella spp*. In Chinese medicine, dried *Gastrodia elata* used to curing the paralysis, high blood pressure, stress, insomnia, etc. In generally, *Gastrodia elata* cultivating at mountain area or opened upland. But, this culturing system was very low productivity because of inappropriate environmental conditions so as temperature, humidity, etc. These experiments were conducted to improve the *Gastrodia* elata productivity by change the cropping system.
For improve the culturing environment condition, we cultivated in plastic house, which covered with polyvinyl, lagging material and shade net, instead of opened upland. Two stairs of culturing bed and irrigation system was established in the plastic house. To find the optimum media composition for growing the *Gastrodia elata* and *Armillariella spp*., we are mixed deciduous tree sawdust and waste cotton to several rates. Bed culturing practice, several medium materials such as oak log, deciduous tree sawdust, sand, upland soil and silt loam was arranged different combination, and planting the spawn (*Armillariella* spp.) and young root of *Gastrodia elata*. All experiment plot management same methods and harvest two years later.
In container cultures, the best media composition for growth of G. elata and Armillariella spp. was planted only deciduous tree sawdust medium. Mean weight and number of G. elata was decreased in inverse proportion the amount of waste cotton. Because the waste cotton have a high capacity of water holding for a long periods, young root of *G. elata* was decomposed easily.
In bed culturing at plastic house, the most effective oak log arrange methods was following as the length of oak log was 30cm, distance of each oak logs was 20cm. Medium composition was silt loam + oak log + deciduous tree sawdust. In this plot, the yield of the *Gastrodia elata* was 31.3kg/3.3. This yields was improved 3.7 times and the yield index of bed culturing method was improved 4.8 times compared to open upland soil culture.

PS7-602-0328

Reduction effect the production cost of *Flammulina velutipes* by re-using of the used media

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Mushroom industry is one of the most growing industry in the Korea. *F. velutipes* began to cultivate in large quantities from 1992 in our country. *F. velutipes* is produced much secondarily by 32,796 metric tons that is 20.9% of internal mushroom total output 156,599 metric tons.
In generally, mushroom cultivation using bottle have a several profits which automation of work, short cultivation period and low contamination rate, compare to bed cultivation. In this point, we have a several questions. Is it possible the reuse of used-media for culturing *Flammulina velutipes?* How can I put the used-media? How about quantity or quality when mixed the used media? Therefore, the purpose of this experiments were productivity elevation of *F. velutipes* and investigates the curtailment effect of media material expense.
Strain that use for this experiment is "Paengi 2 ho"(Flammulina velutipes). The material for media formation was used needle-leaf tree sawdust, media that finish 1th cultivation of F. velutipes, corncob meal, and rice bran, wheat bran by nutrition. We made 14 kinds of media differently and inoculate the F. velutipes and checked the spawn growth speed, fruit body quality and quantity.
Two nutrition agents, which is rice bran and wheat bran, did not affected the incubation period, but the effective stem number, quality and quantity of fruit body was better at rice bran than wheat bran. The fruit body quality produced at mixed 20% of used media (needle-leaf tree sawdust 60% + used media 20% + rice bran 20%) was similar to control plot (needle-leaf tree sawdust 80% + rice bran 20%), and yield was 142.2g that improve 10% more than control plot 130g. But, according as the used-media mixing amount increases, quality and quantity of fruit body became low remarkably. Therefore, the most suitable used-media mixing amount was 20% and quality same, and quantity improved 10% and material expense could reduce 27%.

PS7-603-0330

The Sexuality of *Cordyceps militaris* and Production of Cordycepin by Hybridization of Monosprorous strains

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Many species in the genus of *Cordyceps* were used as traditional medicine for several hundred years. Among them, *Cordyceps* militaris was recently developed as functional food in Taiwan. C. *militaris* was characterizes with the production of cordycepin that was found to be functional in antitumor and antivirus. The purpose of the present study is to breed a high cordycepin production strain through mating of mono-ascosporoes cultures and the mating types of C. *militaris* were detected in the same time. Ascospores discharged from cultivated stromata were isolated individually from the surface of agar plate and grown in PDA slants at 25?. The pigmentation of the monosporous strains can be separated into three types as white, light-yellow and deep-yellow. The growth rate was also different among these types that indicated the meiotic segregation of the strains. HCl-Giemsa stain revealed that all the strain before and after a all possible mating were single nucleated in hyphae and phialoconidia. Flow cytometric analysis on the conidia suspension stained by acridine orange indicated that the mating occurred for some mating and the intensity of DNA demonstrated in two forms which were suggested to be diploid and haploid strains. To confirm the mating type of C. *militaris*, primers of MAT-1 and MAT-2 were designed for PCR. HPLC were employed to analysis the production of cordycepin of the strains before and after mating and high production strains were thereafter selected.

PS7-604-0337

Isolation of monokaryon from asexual spore of *Taiwanofugus camphoratus* and breeding of high triterpenoid strains.

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Taiwanofugus camphoratus Wu et al. contained high triterpenoids and other physiologically active compounds were used as a precious herb in Taiwan. The culture of *T. camphoratus* was grown in PD agar plate, and the agar-slab containing mycelium and asexual spore was spread over agar plates. After 5~8 days, the germinating asexual spore was pick up by a aseptic syringe needle under dissect microscope. The germiling of the asexual spores was transferred to PD agar plate for further growth. A small portion of the mycelium with small piece of agar-slab was transferred to a slide and incubated for another 7-10 days under 25?.After the mycelium was spread over the slide, the agar-slab was removed and the mycelium on the slide was stained by HCl-giemsa. It is obvious that the clamp-connection was disappeared through the hyphae of the single-asexual-spore isolates and all the septate hypha containing only one nucleus. It suggested that the isolates were monokaryons. The mating reaction of isolates are now under investigation. To obtain high triterpenoid strains, cultures of dikaryon and monokaryons were grown on cellophane PD agar for 21 days at 25?. The mycelium part and medium were separately dried. Triterpenoid contain was analyzed by TLC and HPLC. The study will continue to detect the triterpenoid contain in monokaryon and their mated dikaryons, thus high production strain triterpenoid was able to be selected.

PS7-605-0358

Cultivation properties of termite mushroom

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Termitomyces spp. are widely distributed in the tropics of Asia, Africa and South Pacific. They are well known as highly valued edible mushrooms in the regions, but cultivation technology has not been developed yet. They have symbiotic relationships with the fungus-growing termites, and mainly decompose the plant material of the nests. The termites consume the decomposed materials and fungal biomass. Therefore, the fruit bodies arise only from nests deep underground. In the present paper, we introduce a few preliminary cultivation properties of Termitomyces sp..
Fruit bodies were collected from Tenganan province in Bali Island, and tested strains were isolated from the tissue and maintained as stock culture. Accoding to the morphologic characteristics, these specimens were identified as *Termitomyces eurrhizus* (Berk.) Heim: Pileus 7-10cm wide, grayish brown, campanulate then almost to plane with umbonate perforatorium, surface smooth and viscid when moist. Stipe 3-5.5 x 1.3-2.5cm, surface white, attenuated towards the apex. Context fleshy, white. Gills white to pinkish gray. Spores 7-9 x 5-6 um, ellipsoid.
The pseudorrhiza was connected to living termite nest, and many workers, soldiers and larvae were observed inside. The host termites were identified as *Odontotermes* sp.. The white tufts of *Termitomyces* and grayish green tufts of *Xylaria* were observed on the surface of nests. The C/N ratio and pH of the fungus garden (termite nest) were 22.5-33 and 3-5 respectively.
Mycelial growth speed was very low on the PDA medium. Moderately good growth occurred on Hamada medium, and grayish-white colony with little aerial hyphae was presented. For optimum growth, initial pH of the medium was adjusted to pH5.0, not changed during the incubation. The optimum temperature was ranged in 25-30C. Light irradiation depressed the mycelial growth. Ammonium tartrate and urea were well utilized as nitrogen source in synthetic medium. No conspicuous differece in the growth and density of mycelium could be observed between the natural additives (wood or grass oriented).

PS7-606-0362

Using of the Spent *Pleurotus ostreatus* Substrat Based on Salt Cedar Sawdust, in the Ruminant Feeding

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ZERI Sustainable Community Santa Fe, New Mexico, USA has started with serious investigation in the filed of using different tree materials, originated from Santa Fe and Rio Grande valley region, for different purposes. One of the most important goals of the study was finding solution for the serious ecological problem generated by abundance of the population of the salt cedar tree. Likewise, it was initiated investigation and collection of the forest mushroom from the region of New Mexico, in order to create specific mycelia collection. The cultivation of lignocellulolitic mushroom strains, including *Pleurotus ostreatus* (wild types) on the specific tree material, mainly on salt cedar tree, already had been finalized with positive results. Using spent substrat of P. ostreatus after harvesting period like component in ruminant feeding was the main subject of this project.

The results, which are presented in this article undoubtedly confirm some modification of substrata composition during mycelia growth. Most significant modification was observed in the nitrogen content: from 0,39% at the beginning of the mushroom life cycle to 0.19% at the end of the fructification period. Thes data clearly illustrate activities of mushroom enzyme complex.

Content of neutral detergent fibre and hemicellulose was lower at the end of growing cycle from 2.62 to 3.08% that is result of *P. ostreatus* enzyme complex activity (cellulaze, hemicellulaze, celobiaze, ligninaze, etc.). The enzymes influence on better degradation of lignocellulolitic complex.

Quantity of acid detergent fibre, acid detergent lignin and cellulose (difficult degradable complex) was not significantly modified during mushroom growing cycle. Moreover, digestibility of the substrat dry matter after ending of mushroom growing cycle was lower (8.94%) by comparing to the digestibility immediately after substrata inoculation (10,84%).

The obtained results show possibilities of utilization of spent *P. ostreatus* substrat produced by salt cedar sawdust in the animal feeding. The hypothesis for using spent substrat like component of silage production with corn was created, at the base on this investigation.

PS7-607-0365

Moriniafungin, a potent antifungal sordarin derivative produced by the endophytic fungus *Morinia pestalozzioides*

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During a screening of fungal metabolites with antifungal activity, a novel sordarin derivative, moriniafungin, was discovered from cultures of an endophytic isolate. The producing fungus was cultured from Sedum sediforme collected in Spain and was identified as *Morinia pestalozzioides*. For the detection and isolation of moriniafungin a highly specific bioassay was employed consisting of a panel of *Saccharomyces cerevisiae* strains containing chimeric *eEF2* for *Candida glabrata, C. krusei, C. lusitaniae, Cryptococcus neoformans,* and *Aspergillus fumigatus* as well as wild type and human *eEF2*. Moriniafungin exhibited an MIC of 6 µg/ml versus *Candida albicans* and IC50's ranging from 0.9 to 70 µg/ml against a panel of clinically relevant *Candida* strains. The compound inhibited in vitro translation in the chimeric S. cerevisiae strains at levels consistent with the observed IC50. Moriniafungin showed the broadest antifungal spectrum and most potent activity of any natural sordarin analog identified to date. Chemical characterization indicated that moriniafungin contains a 2-hydroxysebacic acid residue linked to C-3' of the sordarose residue of sordarin through a 1,3-dioxolan-4-one ring. An additional set of fungal isolates including strains of another *Morinia* species, *M. longiappendiculata*, and a number of anamorphic Amphisphaeriaceae taxonomically related to *Morinia* were tested for production of moriniafungin in culture. None of these isolates produced the active compound when grown in parallel conditions.

PS7-608-0380

Prospects in use *Leccinum* sect. Scabra as biomonitors of caesium-137 pollution in wood communities

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To determine pollution of fungi by radioactive elements on morphological attributes is impossible. But early to consider this opinion as final. Follows to lead additional researches, directed on search of bioindicators, capable to change morphological attributes at pollution of wood community by radioactive elements. For designation of such species the term biomonitors is offered.
It is known, that caesium-137 is the basic source of an irradiation in the territories, undergone to radioactive pollution of a Chernobyl origin. It is established, that fungi absorb caesium-137 in 10 times more, than isotopes of plutonium 238-240 and in 1000 times more, than strontium-90. As a result of selective accumulation concentration of caesium-137 in fruit bodies can be in 20 times above, than in the polluted wood laying (O-horizon).
In work the method of sporocarps mapping on study plots established in pure wood communities and subjected to pollution by caesium-137 was used. After comparison of fruit bodies on morphological and microscopic attributes measurement of specific activity of caesium-137 was carried out.
Data obtained to the present time testify that representatives of subsection Scabra Pilat and Dermek, relating to the obligate ectomycorrhizal genus *Leccinum* S.F. Gray, are the biomonitors, capable to change morphological attributes, growing in the wood communities polluted by caesium-137. "Atypical" fruit bodies at which has changed as one macromorphological attribute are found – leg squamules became lagging behind and large, and set of attributes – thin round tube pores became wide and angular, and instead squamules the stipe is covered of flakes fur. Thus spores quantity is normal, and microscopic characteristics are in an interval specified for species of subsection Scabra.
The estimation of herbarium samples has shown, that in "atypical" fruit bodies specific activity of caesium-137 in 3,2-3,7 times more than in the fruit bodies collected on not polluted trial areas, that in 1,5-1,7 times exceeds an admissible level in the dry fungi, making according to sanitary norms accepted in Russia for dry mushrooms 2500 Bk/kg. Specific activity in the fruit bodies collected on not polluted trial areas, has made 0,5 admissible levels.
An explanation of this phenomenon should search in features of a Gene structure of the species of Leccinum, which require the further revealing. It is already known, that *Leccinum* – the first genus among basidiomycetes at which representatives the area of ITS1 rDNA is organized with minisatellites participation. In the literature presence of such repetitions connect with intraspecific polymorphism.
This work was supported by grant of St.-Petersburg Government PD061.4-142

PS7-609-0396

Bioconversion of trace contaminants by aquatic fungi

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Trace contaminants found in aqueous environments led to increasing concerns regarding their potentially hazardous effects on human health and the environment, but the knowledge about their biodegradability by microorganisms of aquatic environments is still limited. Technical nonylphenol, a mixture of mainly para-substituted nonylphenol isomers with variously branched side chains, is known to act as a xenoestrogen. Galaxolide (HHCB) and tonalide (AHTN) are polycyclic musk fragrances used in personal care products and were reported to inhibit multixenobiotic resistance transporters in aquatic organisms. Triclosan is an antiseptic compound used in medical and consumer care products, with a reported toxicity for fish and certain algae species. Carbamazepine is an antiepileptic drug known to cause several side effects on endocrine functions in humans. Several fungal strains isolated from surface waters, among them aquatic hyphomycetes and environmentally ubiquitous micromycetes, were used as model organisms to investigate the metabolism of technical nonylphenol, HHCB, AHTN, triclosan, and carbamazepine. Technical nonylphenol, HHCB, and AHTN were converted into various products. Fungal oxidation of technical nonylphenol starts at the branched nonyl chains, leading to hydroxylated nonylphenol isomers and also to compounds with shortened side chains. Biotransformation products of HHCB and AHTN indicate hydroxylation of the parent compounds and also further bioconversion reactions. Triclosan partly undergoes methylation of its hydroxyl group. Carbamazepine was found to resist fungal attack. Structural modifications of micropollutants caused by fungal biotransformation may increase the susceptibility to further degradation by other microorganisms.

PS7-610-0409

Efficient Immobilization of & Increased Manganese Peroxidase Production by the White-rot Fungus LSK-27

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LSK-27 is a recently isolated white-rot fungus with prominent lignin-degradation and textile-dye removal capability. The organism produces manganese peroxidase (MnP) as part of its lignin-degrading component. Since the fungus appears to be a promising organism for industrial applications, improvement of MnP production capacity of white-rot fungi is of utmost importance, therefore the effect of immobilization on the enzyme production was investigated. Two different immobilization materials, stainless steel sponge and fiber sponge were studied not only to increase MnP production but also the organism's lifetime stability. Extracellular lipid peroxidation and glutathione levels were also studied in order to understand the effects of immobilization systems on the morphological stability of the fungus. It was found that immobilization on stainless steel sponge increased MnP production by about 3 fold. Moreover, we demonstrated that extracellular lipid peroxidation and glutathione levels were decreased when immobilization was applied. Thus, stainless steel sponge appears to be a very useful medium for immobilization of white-rot fungus LSK-27, and improvement of MnP production. We suggest that this immobilization method might help decrease the oxidative stress mainly in terms of membrane stability of the fungus based on the extracellular lipid peroxidation and glutathione levels in the culture fluid.

PS7-611-0468

Effect of lignin on biomass and the activity profile of principal ligninolytic enzymes in submerged cultures of *Lentinula edodes*

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There are a few microorganisms in nature with capacity to grow and degrade efficiently lignin. Lentinula edodes is a white rot fungi that depolymerize lignin by a process that involve oxidative and reductive reactions done by lignin peroxidase (LiP), manganese peroxidase (MnP), laccase (Lac) and aryl alcohol oxidase (AAO) enzymes. The aim of this work was to contribute in the understanding of complex process of lignin depolymerization by testing the effect of lignin in the mycelial biomass production and activity profile of principal ligninolytic enzymes (LiP, MnP, AAO and laccase) during incubation of L. edodes, when it is cultivated in synthetic medium with an without glucose.
Lentinula edodes strain IE-105 was evaluated. The fungus was maintained on potato-dextrose-agar at 4ºC. The organism was grown in submerged cultures (4 mL) at 180 rpm in orbit shaker at 25ºC in the dark, using a 20 mL cotton-plugged flask scintillation vials. Mycelium plugs (1 cm diameter) (cut along the edge of actively growing colony of 10 days old) were used as inocula. Treatments were carried out adding 0 and 1 mg/mL of alkali lignin with Mw 28,000 to media (glucose-peptone 40:10 g/L, pH 4.5). Additionally a liquid medium without glucose (1 g/L peptone and 1 mg/mL lignin) was prepared. The solution was sterilized at 120º C for 30 min prior to inoculation. Protein concentration was determined by the method of Bradford with bovine serum albumin as the standard. All assays were done by triplicated.
Lignin enhanced the mycelial biomass when glucose is present in the culture up to 70 % at 22 days compared with control culture. The lignin media without glucose affected the mycelial growth up to 20 % less than control. Lac, LiP, AAO and MnP had a lower activity in lignin-glucose media and lignin alone apparently enhanced all enzymes after 16 days of culture. Extracellular catalase activity (Cat) was measured as oxidative stress sensor. Cat reached the maximal activity (0.23 U mg-1 protein) at 20 days in lignin-without glucose media and probably are not involved with ligninolytic activity. Carbohydrate source is important to fungal growth, but dissolution of lignin-monomers probably might switch the signal controlling growth rate to a faster mode in Basidiomycetes. Therefore we propose that this polymer exert an influence, as greater as a simple carbon source, on ligninolytic enzymes activity profile and biomass production. Nevertheless, it was not possible to know if lignin can be used as carbon source and if that modification affects lignin depolymerization.

PS7-612-0477

The isolation of NOM degrading fungi

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Natural organic matter (NOM), a complex mixture of brown-coloured carbon compounds derived from the decomposition of plant and animal materials, affects drinking water quality and causes problems in water treatment and distribution processes. The white rot fungi (WRF) produce oxidative enzymes which can break down NOM and so have potential in water treatment, thus reducing the generation of sludge and usage of chemicals as in conventional treatment.
This paper describes the isolation and characterisation of the NOM-degrading activity of some WRF. Their cellulolytic and NOM removal capacities were compared with those of a laboratory strain WRF *Trametes versicolor*, a known lignin and NOM degrader.
Fungi were isolated from several habitats including reservoirs and forests, and with the use of selective enrichment cultures using a NOM concentrate. Cellulose and NOM (obtained from the regeneration of the anionic exchange resin MIEXTM used in water treatment) plate assays were used to assess the degradative abilities of the different isolates. Fungi displaying high cellulose breakdown and NOM removal capacities were further investigated in shake flask cultures using fungal pellets. Degradation of NOM was determined by absorbance as colour removal (A446) and breakage of aromatic and conjugated bonds (A254). Enzyme assays for Lignin peroxidase (LiP), Manganese peroxidase (MnP) and Laccase (Lac) were conducted and changes in the molecular weight of UV-absorbing NOM components were determined by high performance size exclusion chromatography (HPSEC).
Twelve fungal isolates were collected from the various habitats, of these three displayed NOM degradation ability. The forest isolates *Trametes* sp and *P.cinnabarinus*, belonging to the Basidiomycetes, were identified as the most effective NOM and cellulose degraders. Removal of NOM from solution recorded as decolourisation correlated well with the breakage of conjugated and aromatic bonds and consequent reduction in the molecular weight of larger NOM components. Enzyme activities were highest during the periods of greatest NOM degradation with MnP having the highest activity for both fungi. *Trametes* sp demonstrated the best NOM removal ability in both liquid and plate culture under the conditions investigated.
In this study the WRF isolates have shown their potential for the decolourisation and degradation of concentrated NOM wastes arising from potable water treatment. The selection and utilization of a suitable isolate for the biodegradation of NOM and its subsequent removal from water sources is of interest, paving way for more 'natural' and potentially more sustainable processes.

PS7-613-0508

Putting fungi to work: mycoremediation of chemically treated waste wood.

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Mycoremediation of waste wood treated with the chemical preservatives is an alternative to disposal in landfills. Filamentous wood decay fungi have chemical mechanisms that degrade but are not specific to lignocellulose, providing the means for degradation of a wide variety of xenobiotics. Novel isolates of Meruliporia incrassata and Androdia radiculosa were screened for tolerance to the chemical preservatives chromated copper arsenate (CCA), ammonical copper quat (ACQ), creosote or pentachlorophenol (PCP). Tolerant isolates were shown to degrade toxic metals and organopollutants and/or degrade wood treated with the toxic materials. High intensity x-ray methods were used for the nanoscale chemical analysis of fungal remediation of the environmental toxins. The methods provide important information about metal transformation and chemical forms of toxins during remediation, including microXANES for the detection of highly reactive oxidation states of chromium and arsenic, 2-dimensional images of a solid sample with micrometer resolution, and x-ray microtomography for 3-dimensional analysis and bioimaging of the interior of treated wood.

PS7-514-0561

Poroid Mushrooms For Pulp Production

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Production of pulp from wood is a relatively great energy consuming process and wastes that are produced during the production processes become a source of environmental pollutions. In nature, there are numerous types of fungi known as wood degraders, which degrade the wood components at different capacity. The lignin-degrading fungi that degrade lignin component of wood may or may not attack cellulose. Fungi that are good as lignin degraders but poor in utilize cellulose should be benefiting if they are used in pulp production industry. Isolates of 113 poroid mushrooms, collected from tropical rainforest were screened to determine their potential use to degrade lignin materials from wood in pulp production. Evaluation on the isolates degradation ability began with qualitative detection for cellulolytic activity. Of all the tested fungi, 70 isolates showed relatively low or no cellulolytic activity. Then, when these fungi were grown on media for the qualitative ligninases detection, 19 isolates showed having relatively high rates of the enzymes activity. Quantitative assay on enzymes activities were also determined. The results indicated that cellulases activity were in a range of 1.6-6.7X10-2 Uml-1 day-1. Ligninases activity assay in the 19 isolates indicated the present of laccase activity, in a range of 2.5x10-3 -1.04x10-2 Uml-1day-1; however no activity of lignin peroxidase or manganese peroxides were detected. Degradation tests on Acasia mangium wood chips showed the reduction of the chips weight after two weeks inoculated with the fungi isolates were 24.3% to 43.8%. Amongst the isolates that reduced the chips weight at the high percentage were of *Trametes versicolor, Ganoderma sp, Pycnoporus coccineus*, *Haxagonia* sp., *Phellinus* sp., *Microporus* sp. and *Fomes* sp

PS7-615-0597

Evaluation of oyster mushroom growing on woody blocks

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The primary role of wood-destroying fungi is to decompose wood, to humificate it and to restore it into an organic matter cycle. For forestry practice, it is a primary interest to transform a waste wood in forest stands for useful substrate in relatively short time. The oyster mushroom *Pleurotus ostreatus* (Jacq.)P.Kumm.is a native, commonly widespread mushroom in Slovak forests, important like a decomposer of hard and soft wood of various deciduous trees. Its fruitbodies are very popular for a gourmet utilization and their medicinal value is well known too.
The research presented was focused on the observation of beech and aspen wood decomposition by oyster mushroom activity under different environment conditions – forest stand, open area exposed to the south and to the north.
The preliminary results showed through evaluation of biological efficiency B.E. (Stamets 2000) the fastest growing and decomposition of wood at the south exposed open area. The values of B.E. reached in aspen wood 10,23% and in beech wood 9,33% after two years of the experiment.
The growing conditions in beech wood seems to be better at the north exposed open area and in the forest stand. The better conditions for growing of the oyster mushroom in aspen wood are at the south exposed open area.
It is necessary to observe the intensity and rate of wood decomposition under various conditions still three years (Sharma and Jandalk 1985, Pavlik 2005) for evolving a serious plan of optimal processing of waste wood under various conditions.
Acknowledgement: The authors thank the Slovak Grant Agency for Science VEGA for a financial support of the research (grant No. 1/1328/04)
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PS7-616-0608

Bioconversion of agro-wastes by *Lentinula edodes*: the high potential of viticulture residues

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Lentinula edodes has been traditionally cultivated on hardwood logs in order to obtain fruiting bodies for human consumption. However, this cultivation system represents a limiting factor and potential danger to the environment due to the slow growth rate and overuse of the oak. Thus, efforts to develop a more efficient, faster, and more reliable production system have focused on the use of alternative substrates such as straw of different cereals and vineyard pruning. The first are widely used in the cultivation of *Agaricus bisporus* and *Pleurotus* spp. In contrast, vineyard pruning, which has great potential for the mushrooms productions, is practically never used. Mexico is a leader in the production of *A. bisporus* and *Pleurotus* spp. mushrooms in Latin America, with a reported production of ca 40,000 tons for the year 2002. In contrast, only 30 tons of L. edodes are generated per year. The aim of the present study was to evaluate the efficiency of bioconversion of some abundant lignocellulosic by-products for shiitake cultivation.
Four strains of *L. edodes* were evaluated through solid-state fermentation (SSF) of vineyard pruning (VP), barley straw (BS) and wheat straw (WS). Biological efficiency, proximal composition and energy value of the fruiting bodies, as well as substrate chemical changes after harvest, were determined. The shortest primordium formation time (28 d), highest biological efficiency (93.25%), highest yield (37.46%), and shortest production cycle (6 d) were observed in VP. The fruiting bodies obtained from VP had high energy value (379.09 to 392.95 Kcal), high contents of protein (12.37 to 17.19%), carbohydrates (75.26 to 82.22%), minerals (3.26 to 5.40%), but low contents of fat (1.82 to 2.15); these were similar to those reported for the conventional substrate. The substrate chemical composition, due to SSF by *L. edodes,* varied depending on strain, availability of the different fiber fractions, and changes that took place during digestion and fungus growth. Initial hemicellulose, cellulose, and lignin concentration varied among the substrates. After SSF, hemicellulose decreased in all substrates, the cellulose increased on WS and decreased in the rest of the treatments. Lignin decreased after SSF on WS, and BS, but its concentration increased on VP. The variability observed in the degradation capacity of lignocellulosic components was influenced by the substrate's nature, environmental factors, and genetic factors among strains. The vineyard pruning has great potential for shiitake production due to its low cost, short production cycles, and high biological efficiency.

PS7-617-0674

Selection xylanase and glucanase *Produced Aspergillus* and Factors Affecting Enzyme Production in Solid State Culture

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A total of 154 isolates of *Aspergillus* isolated from 42 samples collected from various locations in Thailand were screened for ?-xylanase and ?-glucanase production without alfatoxin formation on solid state culture using 5 g of rice straw and 5 g of rice bran as raw material supplemented with (w/w), 0.1% CuSO4.5H2O, 5 % KH2PO4, 10% corn steep liquor and 0.5 % yeast extract. It was found that isolate ASKU21 that was identified as *A. foetidus* could produce high level of the enzymes. The effect of rice straw and rice bran ratio on enzyme productions was studied. It was found that *A. foetidus* ASKU21, grown on a ratio of rice straw and rice bran at 10:2, produced the highest ß-xylanase and ß-glucanase. Factors, cultivation time, moisture content, spore inoculum's size and corn steep liquor content affecting enzyme productions were optimized using response surface method in solid state fermentation using rice straw and rice bran ratio at 10:2 as substrate. An inoculum's size of 5?105 spores/g substrate, initial moisture content of 70%, cultivation time of 5 days and 4.8 g/g substrate of corn steep liquor were predicted as the optimized level for ?-xylanase and ?-glucanase, yielded 6060 and 3065 U/g solid, respectively.

PS7-618-0712

Isolation, Selection and Optimization for Xylanase Production from *Aspergillus niger* Isolated from Soil in Thailand

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Xylanase producing fungi were isolated from soil in Thailand. Thirty strains of fungi different in macroscopic morphology were isolated. Among these 30 strains, The fungus no. 27 was selected as potential xylan-digesting fungi. The xylanase activity of the fungal strain no. 27 was determined comparing with a reference strain, *Aspergillus foetidus* TISTR 3159 by determined xylanase activity in liquid cultivation. At pH 6, fungi no.27 produced xylanase activity of 33.82 U/ml. The optimum cultural conditions for xylanase production by fungi no. 27 were studied. The maximum xylanase production was obtained in a medium containing 30 g/l corn cobs as carbon source, 0.3 g/l urea, 0.25 g/l proteose peptone, 0.05 g/l yeast extract and 0.3 g of (N)/l (1.4 g/l) of (NH4)2HPO4 as nitrogen sources , 0.2 g/l KH2PO4, 0.3 g/l CaCl2.2H2O, 0.3 g/l MgSO4.7H2O and 2 ml/l Tween 80 at the initial pH of 6.0, a rotation speed of 200 rpm, incubation period of 6 days, and the temperature of 30∞C. In the presence of selective medium, the maximal xylanase activity was seven times higher than that in standard medium. The maximum xylanase and cellulase activity of fungi no. 27 were detected after 5 days of cultivation at 236.53 U/ml and 0.22 U/ml, respectively. The maximum xylanase and cellulase of reference stain were obtained on the 5th day of incubation at 211.90 and 0.28 U/ml respectively. The fungi no.27 was identified as *Aspergillus* niger.

PS7-619-0714

Pigmented basidiomycetous yeasts are the perspective source of carotenoids.

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Carotenoid pigments are widespread among the different organisms on the Earth. They are frequently found in plants, animals and microorganisms. Those compounds are very important due to specific structure of carotenoids i.e. presence of double dangling isoprenoid bonds. For many organisms carotenoids function free radicals extinction being strong antioxidant. Carotenoids are widely used in agriculture (as pigments for egg yolks), aquaculture (salmon fish feeding) and medicine (cancer atherosclerosis therapy). Pigmented yeast fungi are interesting for the biotechnology. Normally these yeasts are able to assimilate a wide spectrum of different carbon sources at relatively high temperatures and form large biomass. Searching for the new strains producing carotenoid pigments that could be perspective in further selection was the aim for this screening.

About 200 isolates related to different anamorphic and teleomorphic taxa (*Cryptococcus, Dioszegia, Rhodotorula, Rhodosporidium, Sporobolomyces*, and *Sporidiobolus*) were studied. All the strains were grown in rotary shacking flasks in liquid medium (glucose; peptone; yeast extract; potassium monophosphate; potassium diphosphate; magnesium sulfate; sodium chloride; ammonium sulfate; calcium chloride). Pigments were extracted from the cell biomass using acetone. At the first step the level of carotenoids was measured (evaluated) photometrically. The wavelength was optimized to absorbtion maximum of beta-carotene and toruline. For the representative strains showed relatively high carotenoids level the HPLC was used. The later results are combined and summarized in following table.

Taxonomical position of the studied strains	Toruline, mkg/g	Torulorodine, mkg/g dry cells	Beta-carotene, dry cells
Rhodotorula glutinis	101.4	6.1	52.2
Rhodotorula minuta	38.8	4.6	4.4
Rhodosporidium sphaerocarpum	25.6	3.7	74.6
Sporobolomyces roseus	81.6	66	11.8
Cryptococcus victoriae	5.8	5.2	2.4
Rhodosporidium diobovatum	124.2	17	4.1
Cystofilobasidium capitatum	24.9	26.7	89.9

The specifity of the studied strains was the absence of licopene among the analyzed pigments. In the most cases dominating pigments were following: toruline, torulorodine and beta-carotene. The highest level of beta-carotene was indicative for the members of *Sporidiobolales*. Anamorphic *Cryptococcus* strains belonged to Tremellales and Filobasidiales demonstrate low carotene level. The best result obtained for them – 2.4 mkg/g (Cryptococcus victoriae). Screening for the yeasts producing compounds of biotechnological interest, for example, carotenoid pigments could be very fruitful in the case of different taxa studied.

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PS7-621-0734

Biotechnological Potential of Keratinophilic Fungi and their Secondary Secondary Metabolites

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Keratinophilic fungi constitute an important ecological group of microbes which are able to colonize and degrade structurally very hard and stable animal protein – the keratin. Keratinophilic fungi are distributed in wide range of habitats including river streams, school, parks, forests, pastures, etc. These can be differentiated by several traditional parameters including microscopic and colony morphology, nutritional requirement, growth temperature, pigmentation, hair perforation, and mating reactions etc. Molecular techniques for identifications of these can be an added tool. Several of these fungi have known teleomorphs in order Onygenales of Ascomycetes and majority of them are the representative of families Arthrodermaceae, Gymnoascaceae, Myxotrichaceae, and Onygenaceae.
Keratinophilic fungi are of great importance for three main reasons. Firstly, these fungi play a very important role in ecosystem functioning and degrade a major portion of soil keratin. Secondly these fungi are potential producer of industrially important secondary metabolites. Thirdly, they are very important medically. The need of an extensive survey of these group of fungi from unexplored areas and exploitation of their ability to produce secondary metabolites is expressed. The need of culture collection of this group of fungi is highlighted.

PS7-622-0791

Thermomyces lanuginosus isolated from Thailand: high thermostable xylanase production and characterizations

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Xylanases have significant current and potential uses for paper and pulp, animal feed, food, and biofuel. This paper describes isolation of local strains of *Thermomyces lanuginose* in Thailand and their characters on xylanase production. Eighty-seven isolates of *T. lanuginosus* were isolated from samples collected from various ecological systems at different geographical regions of Thailand. All strains could produce cellulase-free xylanases with diversified ability on productivity and thermostability. Sixteen strains were found to be produced high ?-xylanase activity in a range of 100-134 units/ml when the culture was grown on xylan medium at 45?C for 5 days. There were 4 strains having half-life of xylanase activity at 70 oC in pH 6.0 of crude enzyme in a range of 201-266 minutes. Crude enzyme produced by *T. lanuginosus* THKU-49 showed the highest thermostable ??xylanase having half-life at 70 oC 266 minutes with 57 units/ml of ?-xylanase activity. The xylanases from *T. lanuginosus* THKU-2, THKU-9 and THKU-49 were purified to homogeneity with specific activity of 430, 360 and 552 unit/mg, respectively. The molecular weight of purified enzymes estimated by SDS-PAGE was 24.9 kDa. Optimal temperature for these purified enzymes was the same at 70 oC. Half-life at 70 oC in10 mM phosphate buffer pH 6.0 of the purified xylanase were 1,160, 283 and 1,226 min, respectively. Comparison of xylanase gene sequences of lower and higher thermostable ??xylanase producing T. lanuginosus showed amino acid in positions 76, 96 and 145 of xylanase molecule were different.
A strain *T. lanuginosus* THKU 56 showed the highest production of insoluble xylan degrading enzyme activity with the higher pH and thermal stability when the culture was grown in submerged culture using corncob as substrate. A central composite design was used to optimize the fermentation medium with regard to medium component. The medium for the production (41 g.l-1 corncob, 24 g.l-1, 5.0 KH2PO4 g.l-1 and Tween 80 0.3 ml l-1) yielded 526.7 units.ml-1 within 5 day of cultivation at 50 °C under shaking condition.

PS7-623-0816

Decolourising of textile dyes by laccases of *Pleurotus ostreatus* grown in submerged fermentation

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The textile industry releases during manufacturing and usage a complex mixture of polluting recalcitrant chemicals, such as phenolic compounds into the water streams. One interesting approach is to promote the biodegradation of these man-made compounds in wastewater treatment plants. The use of enzymes for treatment or degradation of complex compounds has increased for being highly efficient and selective catalysts in biochemical reaction at environmental condition. In this research, the decolourising capability of an extract with laccase activity (EE) of *Pleurotus ostreatus* grown in submerged fermentation was investigated.
Pleurotus ostreatus was grown in a culture medium containing glucose, yeast extract and trace elements in Erlenmeyer flasks (125 ml) containing 50 ml of culture medium. Further cultivation was carried out at 25°C for 19 days on a rotary shaker at 120 rpm. The culture was filtered and then centrifuged at 20 000 x g for 10 min at 2°C and the supernatant collected as the EE. Laccases activity was evaluated using 2-6 dimethoxyphenol as substrate. The dyes used were; remazol marine, remazol brilliant blue, dianix marine, dianix black, remazol brilliant red, remazol intense red, remazol golden yellow, coomassie brilliant blue, basic red 9, diazine green S, bromophenol blue, methylene blue, methylene red, toluidine blue O and bromocresol green. Dye decolourising was assayed using a mixture containing 950 mL of dye (1 mM in 0.1 M phosphate buffer, pH 7.0) and 50 ml of EE, which was incubated at 30°C for 48 h. Decolourising was followed by absorbance decrease measured at the highest wave length for each dye.
Decolourising of remazol marine, coomassie brilliant blue, basic red 9, bromophenol blue, methylene red and bromocresol green was observed. It is shown that the EE can be used to decolourising wastewater from the textile industry. Experiments to determine the capability of decolourising and biodegradation of different dyes by the EE at different conditions of pH and temperatures are underway in our laboratory.

PS7-624-0817

Increased production of laccases in submerged cultures of *Pleurotus ostreatus* in the presence of copper

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Pleurotus ostreatus is a white-rot fungus that produces laccases, which are ligninolityc enzymes with potential applications in the dyes, paper or textile industries. In this research the enhanced production of laccases in submerged cultures of *Pleurotus* ostreatus by adding copper to the culture medium at the beginning of the fermentation and at the onset of the exponential phase was evaluated.
Methods. Liquid media containing glucose, yeast extract and trace elements were prepared. The pH of the media was adjusted at 6.0 using NaOH 0.1 M. *Pleurotus ostreatus* was grown in Erlenmeyer flasks (125 ml) containing 50 ml of culture medium at 25°C for 25 days at 120 rpm. All cultures were inoculated with three mycelial plugs (4 mm diam) taken of a colony grown on PDA at 25°C for 7 d. The effect of copper in the laccases production was evaluated by 3 different approaches; (1) adding 0.25 g/l of CuSO4 to the culture medium at the beginning of the fermentation, (2) adding 0.25 g/l of CuSO4 to the culture medium at the onset of the exponential phase, (3) without addition of CuSO4. Samples were taken each 24 h from the third to the twenty fifth d of growth. Laccase activity was evaluated using 2-6 dimethoxyphenol as substrate.
Results. Laccases production of cultures grown in medium containing CuSO4 was 8000 U/l and 37500 U/l after 312 h and 456 h of growth, respectively. Laccases production was 27013 U/l when CuSO4 was added to the medium at the onset of the exponential phase (480 h of growth). Without CuSO4, laccase production was 200 U/l after 144 h of growth and 1086 U/l during the stationary phase.
Conclusion. Laccases production in cultures of *Pleurotus* ostreatus increased 34-fold by adding CuSO4 to the culture medium at the beginning of the fermentation and 24-fold when such inducer was added at the onset of the exponential phase. Since CuSO4 enhanced laccases production we suggest assay the influence of different inducers and culture conditions in the laccases activity.

PS7-625-0818

Production of laccases of *Pleurotus ostreatus* in solid-state and liquid-state fermentation

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Solid-state fermentation (SSF) is the growth of microorganisms on or in insoluble substrates in the absence or near-absence of free water. It is a promising technology for the development of several bioprocesses and products and is a cheaper alternative technology to liquid-state fermentation (LSF). The aim of this research was to compare the production of laccases and biomass of two strains of Pleurotus ostreatus in solid-state and liquid-state fermentation.
Two strains of *Pleurotus ostreatus* were used; 32783 from the ATCC and 3526 from the NRRL collection. A liquid medium containing glucose, yeast extract, trace elements and CuSO4 as laccases inductor was prepared. All cultures were inoculated with three mycelial plugs (4 mm diam) taken from the peripheral growth zone of a colony grown on PDA at 25°C for 7 d. The cultures were incubated at 25°C for 25 days on a rotary shaker at 120 rpm. The SSF was carried out in Erlenmeyer flask (125 ml) containing polyurethane foam (PUF) cubes (0.5 height x 0.5 width x 0.5 depth) as an inert support impregnated with 15 ml of sterile culture medium per each 0.5 g of PUF. Previously, the cubes were washed twice with boiled distilled water and oven-dried (at 60°C) for 24 h and then autoclaved at 15 psi for 15 min. An enzymatic extract was obtained from the cubes by using a Buckner funnel. The biomass was determined by difference of weight of the cubes. The LSF was undertaken in Erlenmeyer flasks (125 ml) containing 15 ml of culture medium. An enzymatic extract was obtained by filtration of these cultures. Laccases activity was evaluated using 2-6 dimethoxyphenol as substrate.
In both strains, the biomass produced was slightly higher in LSF than in SSF. The production of biomass in LSF was similar in both strains, however, in SSF the strain 32783 produced less biomass than the strain 3526. The strain 3526 had similar laccases activity in both fermentation systems. The strain 32783 had approximately 10-fold higher laccases activity in LSF than in SSF.
Our results show that under these culture conditions the FML seems to be a suitable system to produce laccases by P. ostreatus 32783.

PS7-626-0823

Chitinases from the biocontrol agent *Metarhizium anisopliae*

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In filamentous fungi, chitinases have at least two physiological roles, hyphal growth / morphogenesis and nutrient acquisition. In general, fungi produce more than one chitinase and the characterization of chitinase genes and enzymes is an important step toward global understanding of their biological role. In entomopathogenic fungi, as *M. anisopliae*, extracellular chitinolytic enzymes are suggested pathogenicity determinants involved in host invasion. Cuticle is the major barrier to fungal penetration and is composed by ca 30% chitin. Although all M. anisopliae strains analyzed to date are prolific producers of chitinases, the role of these enzymes during host infection is still not fully understood. Our group has undertaken the characterization of the chitinolytic system in *M. anisopliae*, considering synthesis and secretion regulation and the role of specific chitinases on the host-infection process. Glycol-chitin gel electrophoresis of chitin induced *M. anisopliae* (strain E6) culture filtrates showed at least five distinct degradation bands, suggesting possible different chitinases. We analyzed the effects of different carbon sources on chitinase synthesis and secretion. When compared to cultures with chitin as sole nitrogen and carbon source, extracellular chitinase activity was progressively reduced when glucose (0.5% to 2%) was added in the culture medium with 0.8% chitin. At 0.1% glucose, the extracellular activity was not reduced. In addition, the cell-bound chitinase activity was drastically reduced from 0.1% glucose to 2% glucose in the same culture system. We purified and characterized a 30kDa extracellular chitinase (CHIT30) capable of degrading chitin to completion, producing mainly N-acetylglucosamine (NAG) and with an endo-acting activity producing also oligomers from swollen chitin. Having both endo- and exochitinase activities CHIT30 is a potential determinant of pathogenicity since, in nature, such a mechanism would favor a rapid and complete degradation of chitin microfibrils, producing monomers for nutrition and induction of further enzyme synthesis and assisting proteases with cuticle breach. Serum anti-CHIT30 specifically detected this chitinase amongst the five isoenzymes shown in glycol-chitin activity gels. The serum was used to show that CHIT30 secretion is upregulated by chitin, tick cuticle and low concentrations of NAG (0.25%) and is downregulated by both high NAG (1%) and glucose (1%) concentrations. The cloned chi3 gene was assigned to code chitinase CHIT30 in *M. anisopliae* var. *anisopliae*. We showed that CHIT30 was produced at tick cuticle (Boophilus microplus) during fungal infection, suggesting its participation in host penetration. A gene coding for a 42kDa endo-chitinase (*chit1* gene) was cloned based on conserved regions of fungal chitinases. The deduced mature protein has a predicted molecular mass of 42 kDa that is in close agreement with the 45 kDa determined for the *M. anisopliae* chitinase identified earlier. The recombinant protein in *E. coli* was characterized as an endochitinase. We have also cloned and characterized the complete chi2 gene from strain E6, that codes for a putative chitinase of 42 kDa. We are currently constructing overexpressing chi3 and chi2 E6 strains to access their function in Metarhizium.
Supported by grants from: PADCT, CNPq, CAPES, PIBIC-UFRGS, FAPERGS.

PS7-627-0825

Chitinases - Fungal Metabolites

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Chitinase hydrolyses N- acetyl glucosamine (1-4)-,- linkages in chitin and chitodextrins. Chitin is available in large amounts in the biosphere and it is produced over several thousand tonnes per annum. Chitin is most widely found and forms the structural component of cell walls of various fungi and exoskeleton of invertebrates. Chitinases are well known due to their ability to degrade chitin containing cell wall of many fungi. This property makes it most valuable in the field of pest control, pollution abatement, basic and commercial biology. Recently chitinase emerged as new drug target in asthma. The capability of chitinases to degrade chitin makes it promising enzymes in production of value added metabolites from chitinous waste. Due to the important application of chitinase there has been a lot of research in the recent years for enhanced production of chitinases. The use of fungi for the production of commercial metabolites is an age-old process, but it has increased rapidly over last fifty years. Though fungi are morphologically complex organisms, differing in structure at different times in their life cycle have two forms of structure filamentous and pellets. The research has been done with reference to the synthesis of chitinases but little attention has been given on fungal morphology related synthesis of chitinase enzymes. Hence, this investigation is significant in biosynthesis of chitinases which are important in industries.
The chitinases are found in bacteria, plants, fungi, invertebrates and micro organisms. Chitinases produced from bacteria and plants are less stable compared to that obtained from fungi during other metabolite production. Thus, the present investigation concentrates on the morphology related production of chitinases from micro organism's viz., Trichoderma harzianum, Fusarium chlamydosporum and Talaromyceses emersonni. These fungi have been extensively used in the commercial production of a wide range of secondary metabolites, and their morphological type is closely related to metabolite production. In this work, emphasis is on the estimation of morphological parameters, viz., mean hyphal length, mean hyphal growth unit, tip extension rate and mean equivalent diameter relating to chitinases production and to develop suitable kinetic mechanism. The large scale production of chitinases is expensive and uneconomical to make this enzyme available in sufficient quantities. Hence, there is a wide scope for economically viable source of chitinase. The present investigation is being concentrated on effective application of chitinases from selective microorganisms.

PS7-628-0845

Phthalate degradation by mangrove soil fungi

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Phthalate esters are commonly used plasticisers and can be released to the environment. Even low concentration of phthalate esters has been reported to be harmful to human endocrine system. Degradation mechanisms and pathways of phthalates have been consolidated in bacteria while fungal degradation has not been shown. This study reports the first agar plate screening of phthalate-degrading fungi. Twenty fungi were isolated from mangrove sediment collected at various locations at Futian Nature Reserve in Shenzhen, China and subsequently inoculated onto four different agar media in 25‰ natural seawater: (i) SN (12.5 mM (NH4)2SO4, 0.5 g/L KH2PO4, 0.5 g/L K2HPO4), (ii) SNG (SN + 1% glucose), (iii) SNT (SN + 100 mg/L dimethyl phthalate (DMP)), and (iv) SNGT (SN + 1% glucose + 100 mg/L DMP). After 1 week incubation at 25∞C, colony diameter on various media was measured. Ability of these fungi to degrade DMP can be classified into 4 groups: (i) no significant difference in growth between SN/SNT and SNG/SNGT, (ii) comparable growth in all media, (iii) reduced growth in SNT and SNGT, and (iv) enhanced growth in SNT and SNGT. Among the six fungal isolates with enhanced growth on phthalate-containing media, colony diameter of isolates FT405, FT409 and FT502 on SNT was significantly bigger than that on SN, which suggests the ability of these fungi to efficiently degrade and utilise DMP as the sole carbon source. Results of the depletion assay to determine the rate of DMP utilisation and the intermediate products of DMP degradation by these three fungi will be presented.

PS7-629-0848

Screening of wood rot fungi related to biodegradation of dimethyl phthalate and evaluation of its enzymes

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Dimetyl phthalate (DMP) is one of most abundant phthalate esters, which are exclusively used over the world. However, they can easily enter into the environment because the phthalate esters are not covalently bound to the PVC resin. There is a big concern about the release into the environment, because the phthalate esters are considered as potential carcinogens, teratogens, and mutagens to human beings. To reduce the harmful effects of phthalate esters on human body, it is very important to degrade and mineralize DMP from environment. White rot fungi have shown useful oxidative ability to various environmental pollutant including chlorinated compounds, phenolic compounds, and non-phenolic compounds. This research was performed to screen the wood rot fungi related to biodegradation of dimethyl phthalate and to evaluate its enzymes.
This research was performed to screen the wood rot fungi related to biodegradation of dimethyl phthalate and to evaluate its enzymes. Degradation rate was determined using 6 white rot fungi and 6 brown rot fungi. The inhibition of mycelial growth by DMP in PDA was determined by the length of mycelium on PDA. PDA was treated with 0, 250, 750 and 1,250 uM concentrations of DMP. Reverse phase HPLC with C18 column (Zorbax C18 column, Hewlett Packard, USA) was used for the determination of degradation rate of DBP. For the investigation of enzymes related to biodegradation, activities of ligninases (manganese peroxidase, laccase), cellulases (cellobiohydrolase, ,-glucosidase) and esterase were measured.
In the resistance test, white rot fungus, *Cystidodontia isubellina*, showed strong resistance to DMP compared to other wood rot fungi degradations. Degradation rate was high in white rot fungi than in brown rot fungi. The highest degradation rate of dimethyl phthalate was obtained as 50% at 7-day after addition of dimethyl phthalate by *Cystidodontia isubellina* among white rot fungi. In the shallow stationary culture of *Cystidodontia isubellina* with high degradation rate (50%), the highest manganese peroxidase and laccase activities were determined, while cellulase activities were very low.
According to the relationship of degradation rate and enzyme activity, lignin degradation enzymes secreted by white rot fungi might influence the dimethyl phthalate degradation.

PS7-630-0864

Biodegradation and adsorption of phenanthrene by fungi isolated from Thailand

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Phenanthrene is one of organic compounds belonging to polycyclic aromatic hydrocarbons (PAHs). This compound has been found contaminated in the environmental. It represents one of the serious threats to the health of the humans and ecosystems. The objective of this research is to isolate phenanthrene degrading fungi from decayed wood and petroleum contaminated soil. Wood rot fungi as well as soil fungi were isolated from several locations in Thailand. A total of 90 isolates were obtained and subjected to screen for their ligninolytic enzymes activity on the medium containing guaiacol, phenol red, or azureB to which their structures resemble lignin and PAHs. The results showed that 14 out of 90 isolates gave positive results for these indicators. The biodegradation and adsorption of phenanthrene by these isolates were determined by growing them in nitrogen limiting media containing 100ppm of phenanthrene. The results revealed that 6 isolates could reduce 75-98% of phenanthrene within 7-28 days with different types of intermediates, while the other 8 isolates could slightly degrade or could not degrade phenanthrene, but were likely to adsorb phenanthrene on their cell wall. Based on morphology, these fungi were classified in the Phylum Basidiomycota, Deuteromycota and Zygomycota. They all have potential to be used in phenanthrene biodegradation and adsorption in order to reduce phenanthrene contamination from environment.

PS7-631-0869

Optimization of extraction and determination of polysaccharide in *Paecilomyces tenuipes* mycelia

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The objective of the present study was to establish a method for determination and extraction of polysaccharide in *Paecilomyces tenuipes* mycelia.
Three polysaccharide determination methods?3,5-dinitrosalicylic acid method, anthrone-sulfuric acid method and phenol-sulfuric acid method?were studied through precision experiments, sample recovery rate experiments and stability experiments.
The polysaccharide content of *Paecilomyces tenuipes* mycelia was detected by anthrone-sulfuric acid method. One-factor-at-a-time approach, Box-Behnken central composite design and SAS software were adopted to investigate the effects of different extraction methods on polysaccharide content of *Paecilomyces tenuipes* mycelia.
Anthrone-sulfuric acid method was the best method for determination of *Paecilomyces tenuipes* mycelia polysaccharide.
The optimal condition of polysaccharide extraction was: water bath treatment(100?) for two hours and four times replication, and weight of mycelium/volume of distilled water =1:20.
Under the optimal condition, the polysaccharide content of Paecilomyces tenuipes mycelia was 4.12g/100g.
This is the first report on the optimization of extraction and determination of
Paecilomyces tenuipes mycelia polysaccharide. A convenient, reliable determination method with satisfying stability and repeatability was provided for the study of *Paecilomyces tenuipes* mycelia polysaccharide. The results provided a basis for further study of *Paecilomyces tenuipes* mycelia polysaccharide.

PS7-632-0894

Isolation and identification of dioctyl phthalate-degrading fungi by enrichment cultures from soil

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Phthalate and phthalate esters are plasticizers widely used in the manufacture of plastics which impart flexibility to polyvinyl chloride resins. The most commonly used plasticizer is dioctyl phthalate, with a production of around 500 million kilograms per year in North America alone. Phthalate esters are often discharged by the paper and plastic industries during the manufacturing processes into the ecosystem, contributing to environmental pollution. Some of these compounds are considered carcinogens, teratogens and mutagens. To date, studies on the environmental degradation of these compounds have focused only on the bacterial community. In this study, we used enrichment to isolate fungi capable of utilising dioctyl phthalate as a sole carbon source.
Three culture media (M1, M2 and M3) containing the following composition were prepared; M1) 45 ml of mineral salt medium + 5 ml of soil extract, M2) 45 ml of mineral salt medium + 5 ml of soil extract + 1 ml of dioctyl phthalate and M3) 50 ml of mineral salt medium + 1 ml of dioctyl phthalate. All the cultures were grown in 250 ml Erlenmeyer flasks incubated at 25oC on an orbital shaker at 200 RPM. The inoculum was prepared by adding 1 ml of a 1% soil solution to M1. The first enrichment cultures were prepared by adding 1 ml of inoculum to M1, M2, and M3 and growing for 29 days. The second enrichment cultures were obtained by transferring 1 ml of culture from the first enrichment cultures to fresh M1, M2 and M3 media. Strains were identified by amplification of the 16S rRNA gene followed by interrogation of the NCBI database.
Enrichment by serial transfer of a soil inoculated shake flask containing dioctyl phthalate led to the isolation of three strains capable of using this plasticizer as a sole carbon source which were identified by ribosomal sequencing as *Gloeotinia temulenta*, *Trichosporon akiyoshidainum* and *Hypocrea lixii*.
This study demonstrates that a sub-population of indigenous soil fungi are capable of utilising dioctyl phthalate as a sole carbon source and these fungi can be successfully isolated by enrichment through serial transfer into phthalate-containing medium.

PS7-633-0916

Prospective Cultivation Of The Wild Fungus "*Lentinula sp*" On Oak Sawdust In Mexico

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Lentinula sp. is an edible species widely distributed in central Mexico, with the potential for both beneficial use in local communities and commercial application, and although it grows in isolation in the wild people from the communities include it in their diet during the rainy season. The objective of the present research consists of testing its cultivation for the future supply of a species that is as much nutritional as medicinal.
The species was collected in the wild, dried and preserved, separate vegetative and multi-spore cultivations were made, these were placed in the bank of germinating fluid. The spawn was cultivated on sterile pine, oak and cedar sawdust. The evaluated variables were: biological efficiency; rate of production; length of incubation and phenotypical characteristics of the harvested species; bromated analyses were made as well and finally it will be determined if the fungus in question is a new species or a new species or a new recording for the State of Morelos.
The spawn of *Lentinula* sp. (HEMIM-44) grew suitably on the oak sawdust but no mycelial growth resulted with the pine or cedar sawdust. When grown on the oak sawdust carpophores with desirable commercial characteristics were obtained after a long incubation.
The species under study (*Lentinula* sp.) has affinities qualities to the edible Shiitake fungus (*Lentinula edodes*) which has been introduced into Mexico and has been well accepted by the market for the Lentinula sp. Moreover, this fungus, which has a great cultural tradition of consumption in Mexico, could possibly be, in a short time and at low cost, a species that is enjoyed as much at local community as at a national level.

PS7-634-0960

Domestication of Microorganisms (DOM) – A research programme on safety assessment, production and formulation of microorganisms for envirobiotech applications

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Microorganisms can reduce environmental problems, for example in biocontrol to replace chemical pesticides, in plant growth promotion to reduce nutrient leakage, and in bioprophylaxis to prevent toxic compounds from contaminating the environment. Many applications require large-scale fermentor production of microbial inoculants, followed by formulation steps for long-term stability and to ease application at target sites. Safe use of microorganisms requires careful consequence analyses, which for commercial products may be followed by a lengthy registration procedure. Presently, growth of novel biotechnological industries that can solve environmental problems by using microorganisms is held back by a lack of knowledge about fermentation/formulation technologies. Absence of safety assessment systems for microorganisms, suited for decision making by regulatory authorities, is an even more serious obstacle for sustainable development. The research programme "Domestication of Microorganisms" (DOM, www.mistra.org/program/dom/home) provides microbial solutions to environmental problems through cooperation with strategic partners. This is achieved by utilising the metabolic power of the natural microbial diversity through a domestication programme, focusing on safety and formulation to stable products with high efficacy. Through communication with regulatory authorities at early stages of development, the registration process will be facilitated, allowing earlier commercialisation of novel products. Since process and product safety is assessed at an early stage, potential risks for humans and the environment can be minimised. In DOM phase I, advanced fermentation and formulation equipment was acquired and utilised in research on model microorganisms. During the transition to DOM phase II, the focus will be shifted to industrially relevant bacteria, yeasts and filamentous fungi. Programme activities have a solid base of fundamental research on microbial formulation and safety issues. Industrial partner projects and Industrial short-term contracts. DOM welcomes international cooperation with both academic and industrial partners.

1430-1630

SYMPOSIUM 51 - *Finding the missing taxa: the search for fungi in under-explored habitats*

S51IS1 - 0707

Fungi associated with marine wrack

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Wrack, the combined mass of algae and plants washed up on beaches around the world, is an interesting fungal habitat still in need of study. The habitat is simultaneously terrestrial and marine and attracts biological communities with origins in both environments. The wrack environment has many features in common with dung, arising suddenly and uncolonized and presenting a rich source of nutrients for those organisms able to utilize them. Wrack may undergo profound changes as it remains on shore, changing considerably in moisture content and salinity as it is exposed to either rain or drought. Temperatures on the beach may fluctuate greatly compared to those in the water. Biological communities associated with wrack are diverse but perhaps less complex than more purely terrestrial or marine ones. The fungal components appear to be partly marine in origin and partly terrestrial and show the expected division into saprotrophism and symbiosis. The mycota of wrack is not uniform from one locality to another and is influenced by several factors including geography, season, substrate composition and associated biota.

S51IS2 - 0799

Lessons learned from fungi associated with alcoholic beverage production

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Colloquially known as "warehouse staining", the development of dark discoloration on outdoor surfaces such as concrete, brick and wood as well as many other construction materials has been associated with the aging of spirits since the late 19th century. Many affected building surfaces are sun-exposed, and undergo extreme diurnal fluctuations in temperature and moisture availability. We examined a series of developmental stages of specimens obtained from a broad geographic range including North America, Barbados, Mexico, Argentina, Scotland, France, Denmark and Korea. Using single spore isolation techniques and culture on a whiskey-enriched medium, we recovered a number of cultures of highly melanized, slow-growing, thermotolerant, chlamydosporic fungi that appear to serve as the "founding colonists" responsible for the warehouse staining phenomenon. Morphological comparisons of our specimens to herbarium collections show close resemblances to *Torula compniacensis* Richon nom. nud., and *Capnobotryella renispora* Sugiyama. Phylogenetic analyses of our isolates based on nuclear ribosomal SSU revealed a strongly supported lineage comprising several undescribed taxa allied to the familes Mycosphaerellaceae and Amorphothecaceae, closely related to the microcolonial, epilithic genus *Friedmanniomyces*. Physiological studies confirmed the presence and activity of alcohol dehydrogenase; but our measurements of peak concentrations of fugitive ethanol vapour emissions from liquor warehouses consistently indicated levels far below the average sustained concentration necessary to develop the biomass observed based on a hypothesis of carbon utilization. Apart from primary nutrition, we have additionally demonstrated the function of ethanol both as germination activator and as an regulator of heat-shock protein expression. We propose a primary role for ambient ethanol vapour in the colonization biology of this fungus relating to these latter features that exists independently of the contribution of ethanol to nutrition or vector-mediated dispersal. We furthermore suggest that ethanol vapour and perhaps other microbial volatile organic compounds may similarly mediate the colonization biology of other fungi in underexplored, inhospitable habitats.

S51IS3 - 0719

Vertebrate-associated and keratin degrading fungi from northern Canada

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There have been few studies of the mycobiota of the polar regions and inventories of fungi from the Antarctic and Arctic have focussed largely on plant-associated and soil-inhabiting taxa. Little is known about vertebrate-associated fungi and species responsible for degrading keratin in polar environments. To assess the abundance of these fungi and determine the factors influencing their distribution, we inventoried the microfungi from two under-explored, keratin-rich habitats in northern Canada. In the first study, we isolated fungi from soils collected near the dens of arctic fox (*Alopex lagopus*) and from adjacent non-den sites near Churchill, Manitoba. Nearly 100 species representing 40 genera of ascomycetes were identified, including 10 genera belonging to the Onygenales. Keratinolytic Onygenales (species of *Arthroderma*, *Chrysosporium*, *Ctenomyces,* and *Gymnoascus*) are both more abundant and diverse in soils collected from dens, confirming that the fungi present in soils are good indicators of substrate availability. In a second, recently initiated study, we investigated the mycoflora of the fur of polar bears (*Ursus maritimus*) from the Hudson Bay lowlands by isolating the fungi present on female bears and their cubs as they emerged from maternity dens. Over 70% of the 25 genera of ascomycetes isolated from hairs are sterile, and the dominant taxa (over 50% of all isolates) are slow growing, dematiaceous taxa resembling meristematic Dothideomycetes. Only a single member of the Onygenales was isolated from polar bear hair.

S51PS1 - 0408

High throughput fungal culturing from plant litter by dilution-to-extinction

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Recent advances in high-throughput methods for bacterial cultivation have improved strain recovery of slow-growing and previously uncultured bacteria. Some of the most robust and easily applied high-throughput methods are those based on techniques known as "dilution to extinction" or "extinction culturing." The method consists of low-density partitioning of cells or propagules in tubes or microwells and exploits the fact that species diversity observed in microbial isolations increases as inoculum density decreases. We adapted bacterial high-throughput culturing methods to fungi in order to generate large number of fungal extinction cultures. The efficiency of extinction culturing was assessed by comparing it with particle filtration and plating with an automated plate-streaking device. Equal volumes of particle suspension from five litter collections of the temperate New Zealand forest tree, *Elaeocarpus dentatus,* were compared. Fungal extinction cultures were prepared by pipetting dilute particle suspensions of litter into 48-well tissue culture plates containing 1 ml of agar medium/well. The same particle volumes from the same samples were applied to continuous agar surfaces in omnitray plates by automated streaking and yields of fungi diversity from both methods were measured. The taxonomic spectrum of isolates was assessed by microscopy and by sequencing of the ITS region of rDNA.
Species diversity between the two methods was comparable because the same samples were plated. However, extinction culturing significantly increased species richness. Compared with standard plating methods using the continuous surfaces of Petri plates, extinction culturing distributes fungal propagules over large partitioned surfaces. Intercolony interactions are substantially reduced, permitting longer incubation times, and leading to improved colony initiation, discrimination and recovery. The method substantially reduces labour of plating particles suspensions, and less effort is needed to evaluate and recover colonies from fungal isolation plates.

S51PS2 - 0658

A novel widespread subphylum of Ascomycota unravelled from soil rDNA sampling

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Several recent studies have reported many "unknown" or "unclassified" fungal rDNA sequences from environmental samples of diverse origins. These sequences have not been unambiguously classified within a global fungal phylogeny and cannot be directly compared with each other because they all consist of short (300 - 600 bp) fragments obtained from different portions of the nuclear ribosomal RNA array (rDNA). A study by Schadt et al. (*Science* 310:1359, 2003) indicated that a nLSU-rDNA group detected from tundra soil in Colorado (labeled "Group I") was unique, possibly at the subphyla-class level within the Ascomycota. The purpose of this study was two-fold: (1) to link to this clade disparate data sets of "unknown" or "unclassified" fungal rDNA sequences from previously published studies; and (2) to determine the phylogenetic placement of this clade within the Ascomycota using combined nSSU-nLSU rDNA data. We developed Group I specific primers and used a nested PCR approach to amplify and sequence ca. 3 Kb SSU-ITS1-5.8S-ITS2-LSU rDNA fragments from coniferous forest soil. We then used a series of BLAST searches in GenBank to retrieve partial SSU, LSU or ITS sequences from other independent studies that were similar to Group I sequences. We finally conducted phylogenetic analyses using these sequences and representative members of each major Ascomycota lineage.
Blast searches using various portions of the newly produced 3Kb rDNA fragment as a query sequence retrieved many unclassified sequences from a broad range of habitats and geographic origins which were monophyletic with Group I sequences in phylogenetic analyses. Analyses of partial rDNA sequences were unable to convincingly classify this clade within the Ascomycota. Phylogenetic analyses that includes 3 Kb of Group I rDNA sequence and representative members of each major Ascomycota lineage indicate that the Group I clade occupies a unique and basal position in the phylogeny, distinct from the three currently recognized Subphyla of Ascomycota, the Taphrinomycotina, Saccharomycotina, and Pezizomycotina.
With the use of PCR-based techniques and phylogenetic analyses we have discovered a novel Ascomycota subphylum that is widespread in many diverse habitats worldwide. This new subphylum is more closely related to the Taphrinomycotina and Saccharomycotina than to the Pezizomycotina. Correspondingly, we speculate that members of this new subphylum, like most members of the first two, do not produce conspicuous ascomata or other macroscopic structures, and similar to many Taphrinomycotina, are obligate biotrophs or intracellular parasites. This could explain why this subphylum has been overlooked in the past.

S52IS1 - 0676

How host specific are *Mycosphaerella* spp. infecting eucalypts?

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Close to 3000 species of *Mycosphaerella* have thus far been named, with most descriptions being based on the premise of host specificity. Because members of these taxa are notoriously difficult to cultivate, this hypothesis has been left almost unchallenged. With the advent of molecular techniques, however, comparisons between sterile or slow-growing cultures have become easier to perform.
The genus *Eucalyptus* consists of close to 700 species, on which approximately 100 species of *Mycosphaerella* are known to occur. The fact that *Mycosphaerella* has close to 30-odd associated anamorph genera, of which some are very speciose, means that many more species could also represent apparently asexual lineages of *Mycosphaerella*. We suggest that there could be at least as many *Mycosphaerella* spp. on eucalypts as there are currently recognised species of that genus. This would imply that only 14% of the species of *Mycosphaerella* from eucalypts have been described. However, our data also indicate that not all species occurring on *Eucalyptus* are host-specific, as some have also been recorded from obscure, unrelated hosts. In such cases of growth on secondary hosts, *Mycosphaerella* spp. appear to form only a limited number of ascospores, with the sole advantage of onward dispersal in search of their ideal host. This phenomenon is explained by means of the "pogo-stick" hypothesis, which suggests that *Mycosphaerella* spp. and their anamorphs can sporulate on atypical hosts as means of amplifying the chance that inoculum will locate the corresponding ideal hosts. Fructifications of taxa crossing over ecologically in this way usually occur in lesions caused by other *Mycosphaerella* species that are primary pathogens of the affected specific host. This finding suggests, therefore, that in addition to the primarily and secondarily pathogenic, endophytic and saprobic species, some isolations would also reveal non-pathogens of that host.

S52IS2 - 0469

Mycorrhizal fungi and eucalypts - fungal significance in conservation and land management

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Many hundreds of species of fungi form mycorrhizal associations with eucalypts in vegetation ranging from tall wet forest to semi-arid mallee. The majority are ectomycorrhizal fungi. These include predominantly agaricoid genera such as *Amanita* and *Cortinarius*, as well as sequestrate, resupinate, and many other forms of fungi. At least three broad themes emphasize the significance of these fungi in conservation and land management: (1) Diversity and endemism - The fungi can be a significant component of the species biodiversity of a given area of natural eucalypt vegetation. Many of the fungal species may be endemic to regions where eucalypts occur. (2) Function - The eucalypt fungi undertake ecosystem functions attributed to mycorrhizal associations in general – e.g. distribution of nutrients via mycelial networks including capture of nutrients and provision of them to plants and animals. (3) Sensitivity and response to environmental change – Individual taxa and communities of eucalypt mycorrhizal fungi respond to and recover from natural and human-induced changes in different ways. In certain regions of Australia where extremely diminished natural vegetation corresponds with a commitment by local landholders to restore biodiverse native vegetation, specific protocols may be applied to help promote the return of local fungi diversity.
Conservation and management of eucalypt -dominated vegetation is a major issue, particularly in Australia. Consideration of eucalypt mycorrhizal fungi to help develop and apply better decisions and practices is impeded by a poor knowledge base about the fungi. The challenge now is to build upon the impetus created by some recently established public and academic fungi programs in Australia, to coordinate new and classical technologies, and to make available an improved fungal knowledge system.

S52IS3 - 0708

Eucalypts as the natural host for the human pathogenic fungus *Cryptococcus gattii*.

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Cryptococcus gattii is an encapsulated basidiomycete yeast-like fungus with a predilection for the respiratory and nervous system of humans and animals. The distribution of human infections due to this fungus is geographically restricted, non-immunocompromised hosts are usually affected, large mass lesions in lung and/or brain (cryptococcomas) are characteristic and morbidity from neurological disease is high. Human disease is endemic in Australia, Papua New Guinea, parts of Africa and the Mediterranean region, India, Southeast Asia, Mexico, Columbia, Brazil, Paraguay and Southern California. Incidence figures from Australia show a strong rural setting (71% of cases) and the incidence in the Aboriginal population was approximately 5 times that of the non-Aboriginal population. This increased incidence was not associated with overt immunocompromise and is perhaps consistent with high levels of local environmental exposure to *C. gattii*. Environmental isolations, initially from the Barossa Valley in South Australia have established that *C. gattii* has a specific ecological association with several species of eucalypts; notably *Eucalyptus camaldulensis*, *?E. tereticornis*, *E. rudis*, *E. gomphocephala and?E. blakelyi* [all VG I type] and *E. tetrodonta and E. miniata* [VG II type from Arnhem Land]. Three of these species (*E. camaldulensis, E. tereticornis, E. gomphocephala*) have been exported extensively to several of the countries in which human disease due to *C. gattii* has been reported though the association is not exact. Outside of Australia isolations of *C. gattii* have been made from eucalypts in California, Italy, Spain and India. In a recent ongoing outbreak on Vancouver Island, *C. gattti* has now been recovered from multiple species of native Canadian trees, but not yet from any introduced *Eucalyptus* species examined. This outbreak may be attributable to a recent recombination event that has allowed *C. gattii* to colonise new host trees? so additional environmental niches may yet to be discovered.

S52PS1 - 0322

A reassessment of *Phaeophleospora* species on eucalypts.

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Phaeophleospora destructans is a devastating eucalypt leaf pathogen first described in 1996 from 1-3 years old *Eucalyptus grandis* in Sumatra, Indonesia. Since then it has been recorded from Thailand, East Timor, Vietnam and China. ITS sequence data of *P. destructans* on GenBank shows it to be closely related to the type species of the genus *Phaeophleospora*; *P. eugeniae* a pathogen of *Eugenia uniflora* described from Brazil. In order to develop molecular markers to study the rapid movement of *P. destructans* around Asia, several populations were collected from various regions and isolates sequenced using four protein gene regions; ITS, elongation factor 1-alpha, beta-tubulin and chitin synthetase. The combined gene tree showed that *P. destructans* is closely related to *P. eucalypti* but not to *P. eugeniae*. With the recent redescription of the genus *Colletogloeopsis*, morphological characters between *Phaeophleospora* and *Colletogloeopsis* overlap, so what is *Phaeophleospora*? DNA based phylogenies has shown that all sequenced *Phaaeophleospora* spp. from eucalypts and all *Colletogloeopsis* spp. fall in a clade together with *Mycosphaerella nubilosa* and far from *P. eugeniae*. We have acquired the original herbarium material for *P. delegatensis* and *P. lilianae*, species for which no cultures exist, and have, to date, successfully obtained sequence data for *P. delegatensis,* which also falls into the *M. nubilosa* clade. These results highlight the need for a revision of the genus *Phaeophleospora*, raising several alternative hypotheses. Do we synonymise *Phaeophleospora* species from eucalypts with *Colletogloeopsis* or resurrect *Kirramyces* for long spores species and use *Colletogloeopsis* for short species or synonymise all *Colletogloeopsis* and *Phaeophleospora* species from eucalypts, with *Kirramyces* (as it is an older genus than *Colletogloeopsis*)?

S52PS2 - 0210

Fire and Fungi: survival, succession and composition of macro fungal community following fire in eucalypt forest in Western Australia.

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In Western Australia, high intensity fire is used as a management tool to aid regeneration of *Eucalypt diversicolor* (karri) forest following clear-cut harvesting while low intensity prescribed burning is used to achieve community protection and biodiversity conservation objectives. Additionally, from 2003-2005 destructive wildfires burnt 14,000 – 126, 000 ha of eucalypt forest and woodland annually. The associated change in soil properties and the loss of litter and woody substrates has a significant impact on fungal species and fungal community dynamics. A recent study in karri forest showed that a distinct mycoflora inhabited recently burnt forests. Species richness was lower on burnt sites but the composition of fungal communities on burnt sites differed significantly each year for at least 5 years following fire. Species richness increased annually on the burnt sites to be 90% of that recorded on the unburnt sites 5 years after the fire. Like plants, many species of macrofungi have adaptive traits that allow them to survive and/or recolonise rapidly following fire. Up to 40 species were recognised as producing sporophores in response to fire. For several species, the response was immediate and large sporophores developed from subterranean sclerotia within days of fire. Five distinct succession groups of post-fire fungi were recognised. Species replacement took place mainly within soil-inhabiting species, occurring concurrently with the re-introduction of saprotrophic species as the litter built up on site. The concept of using fire mosaics to enhance fungal diversity across a landscape is also discussed.

1430-1630
SUMPOSIUM 53 - ***Epidemiology of Fungal Pathogens***

S53IS1 - 0879
***Paracoccidioides brasiliensis*: ecological and evolutionary aspects**
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P. brasiliensis is the etiological agent of paracoccidioidomycosis, one of the most important systemic mycoses in Latin America. This fungus is thermo-dimorphic growing as yeast forms in the host tissue or when cultured at 35-37ºC, and as mycelium in the saprobic condition or when cultured at room temperature (18-23ºC). The teleomorphic or sexual (meiospore) stage is still unknown, but molecular taxonomy indicates the pathogen belongs to the family Onygenaceae (Ascomycota), recently classified in the newly proposed family Ajellomycetaceae. The habitat of the mycelial saprobic phase of *P. brasiliensis*, which produces the infectious propagula, has not been determined and has been a challenge to mycologists. The fungus is rarely isolated from the environment, the disease has a prolonged latency period, and outbreaks have not been reported. These facts have prevented the adoption of preventive measures to avoid infection.
The confirmation of natural infection of armadillos with *P. brasiliensis,* in a high frequency and wide geographic distribution, has opened new opportunities for the study and understanding of the ecology of this fungus. Besides practical purposes such as mapping the location the fungus in nature, these findings also provide interesting insights about the fungus biology. Armadillos belong to the order Xenarthra, family Dasypodidae, which has existed in South America, since the Paleocene Era (65 million years ago), when it was separated from North America. Because both the armadillo group Dasypodidae and Ajellomycetaceae fungi are ancient organisms that might have been cohabiting in the same continental area for at least 10-20 MYA, one may ask whether the armadillo-*P.brasiliensis* association occurred millions of years before humans appeared or, alternatively, if it only has occurred in recent times as the result of human action. There are several biological features of *P. brasiliensis* that seem compatible with a long existence evolving connected with animal hosts, such as its dimorphism, a poor saprobic sporulation, a restricted occurrence in nature and difficulties in its environmental isolation. Similarly, some features of the disease, such as a tendency to induce chronic forms, long latency, relapses, and male-association (it is much more frequent in men than women) may also be associated with an ancient relationship with armadillos or other animal hosts. (Financial support: Fapesp).

S53IS2 - 1008
A global molecular epidemiological survey shows that the Vancouver Island outbreak strain is closely related to Latin American Cryptococcus gattii VGII isolates
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The aim of this study was to investigate the epidemiology of the VGII genotype worldwide and determine the *existence of strains that are closely related to the VGII strains responsible for the outbreak in Vancouver.*
Cryptococcus gattii is an basidiomycetous yeast that causes life-threatening disease mainly in immunocompetent patients. Four molecular types have been identified: VGI-VGIV. Two subtypes of molecular type VGII have emerged in 2000 as a primary pathogen on Vancouver Island, Canada, becoming quickly endemic, with a high attack rate for humans and animals: VGIIa, the major and more virulent genotype and VGIIb, the minor and less virulent genotype. We compared isolates from Australia, Argentina, Brazil, Colombia, Greece, Thailand, Uruguay, USA and Venezuela, recovered since 1986 with the Vancouver Island outbreak strains via PCR fingerprinting and MLST analysis using 5 polymorphic loci: URA5, LAC1, ACT1, CAP59 and PLB1. The mating type was determined by PCR for the vast majority of those isolates and in vitro mating has been performed using MATa and MATa strains.
We demonstrated that the subtype VGIIa found in Vancouver is also present in Colombia, Brazil, Venezuela, Argentina, USA, Thailand and Greece. Especially in Colombia and Brazil it has been present long before the Vancouver Island outbreak occurred. The subtype VGIIb clustered isolates from Vancouver with the majority of the Australian isolates. MLST typing has confirmed the close relationship between isolates from South America and Vancouver Island. An additional interesting finding is that all the Vancouver Island outbreak isolates are mating type a, while the vast majority of Colombian isolates were mating type a. Brazilian VGIIa isolates are identical with the Vancouver Island outbreak strains. Colombian VGIIa isolates are closely related but not identical to the Vancouver Island outbreak strains. The Colombian VGIIa isolates are avirulent. Mating experiments have shown that the avirulent Colombian MATa strains mate with the virulent VGIIa strain from Brazil and Vancouver. The fact that there are isolates belonging to VGIIa recovered as early as in 1986 in South America indicates that this genotype may have been present for a long time in the Americas rather than being a result of a recent recombination event between a less virulent genotype introduced to North America from Australia and an unknown mating partner as suggested previously. Our results suggest that the VGII genotype may have its origins in Latin America and was spread in the environment until its introduction to Vancouver Island due to an unknown event. It is highly likely that due to the fact that Vancouver Island is outside the usual environmental range of *C. gattii* the fungus has undergone changes in its expression profile after its introduction to North America that has led to a more sufficient generation of infectious propagules, resulting in a widespread proliferation of blastospores into the environment, which in turn has let to the Vancouver Island outbreak.

S53IS3 - 0860

Molecular epidemiology of histoplasmosis: An update

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Histoplasmosis, a systemic fungal disease caused by *Histoplasma capsulatum*, is an important health problem worldwide and a very common infection in endemic regions of North and Latin America. The Ohio and Mississippi Valleys in the USA are highly endemic areas, but Latin America presents high frequency of outbreaks being one of the most common systemic mycoses presenting variable prevalence rates in endemic areas and is widely distributed all over the countries. *H. capsulatum* usually grows in enriched soils, and the human infection frequently occurs after inhalation of dust generated from disturbance of *H. capsulatum* micro niches. The risk of infection depends on the activity performed such as soil excavation, spelunking, construction, renovation, demolition, and cleaning sites sheltering the fungus and the period of soil contact. Histoplasmosis represents an occupational and environmental health problem, and has long been recognized as a common recreational disease among cavers in North America, but recent reports indicate an increase in the number of cases in individuals who engage in other forms of adventure tourism and eco-tourism. Also, people in the countryside of some countries are particularly affected, especially miners, peasants, and guano collectors. Information about the genetic diversity of *H. capsulatum* associated to geographic distribution would be very useful to public health authorities in designing and implementing prevention and intervention strategies based on that information. The epidemiology of histoplasmosis will be reviewed, and the current tools used in the molecular epidemiology of this endemic mycosis will be outlined.

S53IS4 - 0760

National, population-based surveillance of candidemia in Australia with emphasis on disease acquired outside of hospitals

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Candidemia is a serious fungal infection associated with high mortality, prolonged hospital admission and substantial health care costs. Medical practice changes towards more frequent use of home health care have increased the number of susceptible patients outside of hospitals, potentially changing the epidemiology of infection in the community. As part of a nationwide survey, the epidemiology and etiology of candidemia were recorded and the characteristics of infection acquired outside of hospitals clarified.
The Australian Candidemia Study (ACS) conducted a nationwide, population-based, active laboratory surveillance of candidemia from 2001-2004. Clinical data and blood culture isolates were collected.
A total of 1095 incident episodes of candidemia were identified. *Candida* species accounted for 3.4% of significant blood stream infections. The annual overall, and hospital-specific incidence was 1.81 per 105 population, and 0.21 per 103 separations, respectively and demonstrated jurisdictional and institutional variation. Inpatient health care-associated (IHCA) cases comprised 81.5% episodes, 11.6% cases were outpatient health care-associated (OHCA) and 6.9% were community-acquired (CA). Predisposing factors included malignancy (37.1%), antimicrobial agents (77%), indwelling vascular catheters (72.6%) and major surgery (37.1%). Cases associated with ICU-acquisition comprised 183 (20%) instances. Co-morbidities and risk factors were similar in IHCA- and OHCA-candidemia. Intravenous drug use was a major risk factor for CA cases (23.8%). IHCA candidemia was significantly associated with sepsis at diagnosis ($p<0.001$), death within 30 days ($p<0.001$) and prolonged hospital stay ($p<0.001$) compared to cases acquired outside hospital. Species distribution included *Candida albicans* (47.3%), *Candida parapsilosis* (19.9%) and *Candida glabrata* (15.4%). The frequency of *C. glabrata* infection increased over time. Non-*albicans Candida* species caused 60.5% OHCA- and 49.9% IHCA- candidemia ($p=0.02$). Variation in species distribution was observed by geography, ward of hospitalisation and patient age. Overall 30-day mortality was 27.7% with age ? 65 years, adult critical care stay, sepsis syndrome at diagnosis and corticosteroid therapy associated with the highest odds for death.
Candidemia in Australian patients demonstrates variation according to geography, case-mix and different exposure to risk factors. Although it remains largely a health care-associated entity in patients with established risk factors, significant proportion of these cases occurred the outpatient setting. OHCA cases demonstrate many characteristics intermediate between those of IHCA and CA infections. *C. albicans* remains the commonest species but the rising incidence of *C. glabrata* should be considered when informing guidelines for antifungal prophylaxis and treatment strategies.

S53PS1 - 0527

Enigmatic amphibian declines and emerging infectious disease: population genetics of the frog killing fungus *Batrachochytrium dendrobatidis*.

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Amphibian populations around the globe are declining due to Chytridiomycosis, an emerging infectious disease caused by the fungal pathogen *Batrachochytrium dendrobatidis*. First identified in 1998 on frogs originating from Australia and Central America, *B. dendrobatidis* has now been reported on every continent except Asia and Antarctica. Little is known about how the disease has dispersed so rapidly and speculation exists that the fungal pathogen may be endemic and that something has triggered its virulence. To address this question we present the first population genetic comparison of *B. dendrobatidis* isolates. Global diversity is low, there is no apparent amphibian-host specificity, and populations show limited geographic relatedness which supports the recent spread theory. Genetic diversity of *B. dendrobatidis* populations, isolated from mountain yellow-legged frogs endemic to California, predicts at least two introductions and a longer association with some sites than previously predicted. A recombination signature was found in two populations suggesting a sexual phase in the life cycle. This provides an alternative theory for the rapid spread of the pathogen. In addition to the live trade of amphibians the disease may be dispersing via resistant sporangia.

1430-1630

SYMPOSIUM 54 - Fusarium - *New Advances in Taxonomy, Biology and Detection*

S54IS1 - 0637

Genetic Diversity in *Fusarium* from Sorghum and Millet

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Fusarium spp. are amongst the most common fungi isolated from sorghum and millets grown often as subsistence crops in marginal environments. For many years the *Fusarium* isolates from these crops were routinely identified as "*Fusarium moniliforme*" even though relatively few of such isolates were the fungus that is now known as *Fusarium verticillioides*, which is common on maize and produces high levels of fumonisins. Two species described in the last few years, *Fusarium thapsinum* and *Fusarium andiyazi* account for many of the *Fusarium* isolates recovered from sorghum in the United States and South Africa, and *Fusarium pseudonygamai* accounts for many of the isolates recovered from pearl millet. Genetic variation within these species varies, with *F. thapsinum* appearing relatively clonal, especially in the United States. *Fusarium nygamai*, an important pathogen in Australia, is less common in the United States and may be polyphyletic. In West Africa, many of the *Fusarium* isolates cannot be readily assigned to a described species and their ability to produce toxins such as fumonisins and moniliformin is largely unexplored. These isolates exhibit relatively high levels of genetic and genotypic variability as assessed by Amplified Fragment Length Polymorphisms (AFLPs). In Egypt, *Fusarium proliferatum* is the most common species recovered and in both Egypt and in West Africa at least one clearly differentiated species has been identified although not yet formally described. Finger millet from Uganda has an extremely complex *Fusarium* population, with 25 or more species identifiable. Variation in DNA sequences of conserved genes, *e.g. tub*-2, and in AFLP banding patterns also is high. The high levels of both intragenic and intergenic species genetic variation suggests that these crops host many undescribed species. That these species can be recovered from native grasses, subsistence agriculture, and commercial agriculture should provide the opportunity for the study of their evolution under different cropping and environmental conditions.

S54IS2 - 0716

Development of an oligonucleotide array for detection of *Fusarium* species by hybridization of PCR products

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Accurate identification of *Fusarium* species currently presents a challenge to plant pathologists and mycologists. Our goal was to develop a sensitive and specific molecular assay to facilitate direct and simultaneous monitoring of several *Fusarium* species without the need for isolation and morphological identification of pure cultures.

A representative set of 219 *Fusarium* strains was selected from a total of 876 isolates recovered from soybean roots during the spring seasons of 2001 and 2002 from 116 commercial fields in eastern Ontario and Québec, Canada. The other sections of the roots from which these isolates were obtained were frozen before DNA extraction. Close to 700 EF1-alpha sequences were obtained from GenBank, our own isolates, and unpublished sequences provided by K. O'Donnell. Using this database and custom designed software, we designed and developed an EF1-alpha oligonucleotide array to target the main species of *Fusarium*. To validate the assay, digoxygenin-labelled amplicons from pure cultures and infected soybean roots were hybridized to the DNA array with the specific oligonucleotides.

Following partial DNA sequencing of the EF1-alpha gene, eleven groups were found among our samples, namely, the *F. oxysporum* complex, the *F. solani* complex, the *F. graminearum* complex, *F. sporotrichioides*, *F. tricinctum*, the *F. equiseti* complex, *F. proliferatum*, *F. sambucinum*, *F. acuminatum* and *Fusarium* sp. cf. *merismoides*. We designed 71 species or clade specific oligonucleotides to target these groups. The novel EF1-alpha DNA array consistently identified the species from pure cultures, and no cross-reactions with oligonucleotides designed for other species were observed. The assay detected *Fusarium* species directly in soybean roots, including the *Fusarium* species implicated in soybean sudden death syndrome disease in North America. The results using the DNA array assays were consistent with the results from direct isolation from the same roots.

The EF1-alpha DNA array provides a new tool for direct detection of several species of *Fusarium* in the environment, in particular from roots. We found that *Fusarium* species were always present in soybean roots as mixtures, which were difficult to characterize by root plating. We developed an accurate tool to detect a wide range of species, but the significance of the interactions between the species remains to be characterized.

S54IS3 – 0300

Secondary metabolome – the bridge between phenetics and phylogenetics in *Fusarium*

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Each *Fusarium* species produces a specific profile of biologically active metabolites, the secondary metabolome, of high importance for its interaction with the environment. These highly functional characters are of great importance for the co-evolution of fungal species, and are hence very informative and useful in fungal systematics. *Fusarium* species to produce several harmful mycotoxins, e.g. the trichothecenes, zearalenones, fumonisins, moniliformin, and beauvericins and other cyclic peptides. Of the trichothecenes, deoxynivalenol (DON) and nivalenol (NIV) are of major concern and are mainly produced by *F. culmorum* and *F. graminearum* (sensu lato), often with co-production in small quantities of the acetylated derivatives. Other NIV-producers are *F. poae*, and *F. equiseti*. *Fusarium culmorum*, *F. graminearum* and *F. equiseti* also produce zearalenone and its derivatives, whereas *F. poae* is a consistent producer of diacetoxyscirpenol (DAS). Another important trichothecene is T-2 toxin, which, together with DAS, is consistently produced by *F. sporotrichioides*, *F. sambucinum*, *F. musarum*, *F. armeniacum* and *F. langsethiae*, whereas trichothecene production has never been detected in common species such as *F. avenaceum* (moniliformin producer), *F. tricinctum* (moniliformin producer), *F. verticillioides* (fumonisin producer) and *F. proliferatum* (fumonisin producer). None of these nine species produce zearalenones.

Most phylogenetic studies are based on nucleotide sequences of housekeeping genes, which convey little, if anything, about the function of an organism. Many recently described *Fusarium* species were initially discovered by molecular tools, such as phylogenetic studies, followed by a formal description of the species based on morphological characteristics. In some cases additional phenotypic characters (e.g. metabolites) are added to the description, but they are seldom integrated into the systematic evaluation of the data justifying the taxonomic proposal. However, today more and more species are being sequenced increasing the available information on the genetics behind metabolite production. This will illustrated using examples of how bioinformatic studies of toxin genes in several species can bridge phenetics and phylogenetics and be the stepping-stones towards a truly holistic approach to *Fusarium* systematics.

S54PS1 - 0427

Assays for rapid multiplex detection of toxigenic *Fusarium* spp. in cereals and derived products applying DNA array hybridisation and capillary SNP analysis, respectively

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Diagnostic sequence motifs for single species and species groups of toxigenic *Fusarium* taxa were recently (Kristensen et al., 2005) identified from a phylogenetic study based on partial translation elongation factor 1 alpha (TEF-1·) sequences. These sequence motifs were successively used to develop two types of PCR derived multiplex diagnostic assays for cereals and derived products, the FusArray and the FuSNaP assay, respectively. Each assay has some advantages over the other: The FusArray may easily be automated and allows for semiquantitation of the contamination level, but each capture probe motif must include at least two to three specific discriminatory nucleotide substitutions. The FuSNaP assay allows for discrimination of single nucleotide polymorphisms (SNPs), but it requires more sample handling and is strictly qualitative. For both assays, the first step is a "universal" PCR amplification of a part of the TEF-1· from the funga present in the gross DNA extracted from the sample, and the final step is the specific analysis of the population of amplification products. The FusArray is a low density DNA array with capture probes corresponding to the diagnostic sequence motifs, and the DNA is labelled prior to hybridisation. The present version of the FusArray includes probes for discrimination of 14 species and several species groups, it is semi-quantitative, its limit of detection (LOD) is estimated to less than 16 copies of the haploid genome of the target species in the initial PCR reaction, and samples can be analysed within one working day. The FuSNaP assay is based on solid phase purification of the amplification products from the initial PCR reaction, followed by multiplex SNaPshot reactions with subsets of specific SNP primers, where the primers in each subset can be discriminated by a combination of migration (size) and colour (SNP specific labelling). The present version of the FuSNaP assay includes SNaPshot primers for 16 species and several species groups and samples can be analysed within two working days. The taxa targeted by the assays are primarily producers of trichothecene and moniliformin mycotoxins, but both assays may be expanded to target additional taxa. Both assays were validated by analysis of five naturally contaminated cereal samples and comparison with data from morphological analyses and chemical toxin analyses of the same samples. A high degree of correspondence was observed, the assays are more sensitive than morphological methods and exceptions from perfect correspondence were few and may be explained by near LOD infection levels.

S54PS2 - 0311

Origin and Diversity of *Fusarium oxysporum* f.sp. *vasinfectum* (*Fov)* in Australia

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Cotton fusarium wilt is attributable to *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*). Eight races and 10 vegetative compatibility groups (VCGs) have been identified outside Australia. In Australia, cotton fusarium wilt was first diagnosed in 1993 and has spread to most major cotton growing regions. Australian *Fov* behave similarly to Race 6 on the differential hosts, but they are vegetatively incompatible with Races 1-8, and have been designated, VCGs 01111 and 01112. This study was undertaken to determine the genetic diversity of *F. oxysporum* present in agricultural and uncultivated soils and to assess the genetic and phylogenetic relationships between *Fov* and co-occurring non-pathogenic *F. oxysporum*. Putative *Fusarium oxysporum* isolates (based on morphological features observed on carnation leaf agar and PDA plates) were obtained from soils in cotton fields, refuges in agricultural regions, and populations of wild Australian cottons (*Gossypium* spp.). The isolates were genotyped using AFLPs and for selected isolates, four gene regions were sequenced. Based on the AFLP genotypes and gene sequences, five distinct lineages were identified, and designated A to E. Three lineages, B, C, D, fall outside *F. oxysporum* sensu stricto, *i.e.*, they are not sister to *F. foetens*. Lineage B is allied with the *Fusarium moniliforme* complex. Lineages C and D have no allies represented in GenBank. Lineages A and E are sister groups that are collectively sister to *F. foetens*, *i.e.*, are *F. oxysporum* in the narrow sense. Australian *Fov* falls into Lineage A, while Races 1 to 8 are allied with Lineage E. The two Australian VCGs form distinct subgroups within the Lineage A clade. Within each VCG the level of genetic diversity is minimal, nonetheless, 21 VCG 01111 and 7 VCG 01112 haplotypes have been identified. VCG 01112 is largely confined to the area in which it was first diagnosed. One haplotype of VCG 01111 dominates all other fields sampled, presumably spreading from its site of origin. Collectively the data suggest that *Fov* arose in Australia from an indigenous lineage of *F. oxysporum* that is genetically and phylogenetically distinct from the lineage of *F. oxysporum* to which all other *Fov* Races are allied.

S55IS1 - 0773
The value of herbaria in the DNA age
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Biological specimens maintained in today's herbaria contain the wealth of the ages representing life through time and space. If one could release and synthesize the knowledge locked in the millions of specimens collected over the past two centuries, the history of life on planet Earth would be revealed. Some progress has been made toward this end. For example, data associated with the one million specimens in the U.S. National Fungus Collections are all available on-line. Thus, if plant quarantine officials need to know the fungi that have been found on conifers in Siberia and thus may be a threat to the U.S. if raw logs are imported from that region, they can easily search on *Abies*, *Picea* and *Pinus* from that part of the world to determine potential pathogens. For those who want to know where to collect morels and when they will be fruiting, just check for these data on the deposited specimens of *Morchella*. Need to identify a rust fungus on *Gladiolus*? Search out the rust specimens on *Gladiolus* to narrow down the possible taxa. Herbarium specimens are increasingly useful as a source of DNA for studies that reveal the origin and distribution of pathogens. For example, DNA from herbarium specimens was also used to determine the spread of *Phytophthora infestans*, cause of potato late blight, from the Andes to Europe and finally North America. Past scientists have conscientiously documented their research with specimens that are extremely useful to modern science in ways they could not have imagined. We can never go back in time to determine what population of soybean rust first attacked wild plants in China. But we can document today's science for future investigators who may use these specimens in ways we could never imagine. Just as scientists who never heard of an electron microscope or a DNA sequencer placed specimens in herbaria for future generations, today's scientists have the responsibility to document their research with voucher specimens deposited in institutional collections. Every sequence in GenBank should be backed by a specimen so that workers in the future can verify the source of that sequence. Ideally DNA barcodes will be linked to descriptions, illustrations, records of accurate plant host and geographic distribution, all backed by voucher specimens. Documented data are required for sound, repeatable science and to provide a legacy for future scientists with their instant genome machines.

S55IS2 - 0788
MycoBank: linking names to genomes
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Taxon names are crucial to anybody working with biological material as they can be considered as shortcuts to access the associated bibliography. While existing species concepts are changing by lumping or splitting, new species are daily isolated from unexplored environments and described. The accessibility to this nomenclatural and taxonomic information is not always easy because of the diversity and of the lack of availability of some journals. The Index of Fungi database, maintained by Paul Kirk at CABI (UK), was a first successful attempt to provide a web based repository on fungal nomenclature. In 2000, the CBS yeast strains and species database/website was launched and already included a wide range of taxonomically informative data usable in the first online polyphasic identification tool (based on the BioloMICs software). In 2005, we created the MycoBank website, a joined effort of CBS and CABI, combining the best of both systems and databases. MycoBank is not only an easy searchable nomenclatural database. It also allows taxonomists to freely deposit their new species (or any other taxonomic levels) descriptions together with associated data. The basic concept is to link the names with the underlying morphological, physiological, metabolic and genomic data. We strongly believe that the useful information and analyzes tools incorporated in MycoBank will greatly help researchers in many fields (taxonomy, agriculture, medicine, pharmacy, etc). A demonstration of the system will be provided together with a preview of the new tools to be associated with MycoBank and that are currently under development.

S55IS3 - 0570

A global network of genetic resource centres to preserve fungal biodiversity

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Of the known 75.000 species of fungi, 30-40% are culturable with current techniques. Of these, about 18.000 species are preserved in the world's biological resource centres (BRC's). Estimates of fungal biodiversity range from 0.5 to 10 million species; 1.5 million being the generally accepted estimate. This leaves us with 1.2% of the biodiversity available as living cultures, many only known from a single strain. For most other groups of micro-organisms (bacteria, archea, algae, protozoan, viruses, plasmids) the situation is worse. It is obvious that the world presently lacks the required number of systematists to adequately document, describe and culture its biodiversity. Even if these fungi were to be described, no single collection has the capacity to preserve such numbers. Moreover, the actual number of culture collections is declining. It is therefore imperative that world-wide networks get established to preserve fungal biodiversity for future generations.
In recent years the Organisation of Economic Co-operation and Development (OECD) has considered the possibility to establish a Global Biological Resource Centre Network (GBRCN) to facilitate access to genetic resources.
A GBRCN must consider (a) its organization (e.g. minimum requirements for membership, structure of network), (b) the distribution of knowledge and (c) the distribution of material. It has to collaborate with many existing organizations, institutes and facilities, for example WFCC, WDCM, GBIF, Catalogue of Life (Species 2000), Index Fungorum, Mycobank, GenBank. It has to consider the consequences of international treaties and national legislation, (OECD, IATA. CBD) and provisions to prevent intentional and unintentional damage (bioterrorism, pathogens, non-indigenous species).
A GBRCN should provide a means to cope with new challenges like barcoding of micro-organisms and DNA-banks. It should form a material and intellectual infrastructure for life sciences and biotechnology.

S55PS1 - 0549

Cooperation between biological resource centers (BRCs) in the CBD era – A challenge of NBRC and BRCs in Asia

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In the era of Convention on Biological Diversity (CBD), Biological Resource Centers (BRCs) are facing difficulties to obtain biological resources from overseas and provide them for users. BRCs are required to collaborate with each other, especially with those of foreign countries to overcome this problem. As an example of the challenge to this problem, I'd like to introduce activities of NITE Biological Resource Center (NBRC, Japan) and its collaboration with BRCs in Asian region.
In 2004, establishment of "Asian Consortium for the Conservation and Sustainable Use of Microbial Resources (ACM)" was proposed by NITE and agreed by 12 Asian countries. Aiming for practical activities including promotion of research, human resource development and network formation, Memorandum of Understandings (MOUs) have been concluded between NITE and each of the representative institution of Indonesia, Vietnam, Myanmar, Thailand and China.
In a program of the Global Taxonomy Initiative (GTI) (2002-2004), mycologists of NBRC and LIPI (Indonesia) joined together in the taxonomic study of fungi in Indonesia. This collaborative study achieved inventory data as well as an advance in capacity building of mycology in Indonesia. Fungal strains isolated and identified in this study are deposited to both collections of NBRC and LIPI, and are distributed to users according to the Material Transfer Agreement (MTA). For enhancement of culture collection activity of both sides, NBRC and BCC (BIOTEC, Thailand) are collaborating in the following programs: (1) Exchange of strains. The exchanged strains are deposited into the collection of the counterpart, and then become available to users according to the MTA. (2) Cooperative studies of taxonomy of fungi, yeasts and bacteria. After identification and taxonomic studies, the strains are deposited to the both collections and opened to users in the same way of (1).
By these collaborations with overseas BRCs, NBRC is now able to transfer biological resources from some Asian countries to NBRC collection and provide them for users in the manner following CBD. Moreover, these activities certainly contribute to the safe preservation of biological resources by duplicate deposition at different BRCs as well as technical transfer and human resource development of both of the partnered BRCs.

1700-1800
PLENARY 5 – 0750
Mike Wingfield
South Africa
The first recorded examples of epidemic diseases resulting in the destruction of forest ecosystems date back to the beginning of the 20th Century. This coincides closely with a time where trade and the movement of wood and wood products, particularly between northern hemisphere countries, increased substantially. It was also a time when forestry industries were being established in many parts of the world and where germplasm required for the establishment of plantations was moved between countries, without control. Diseases such as Dutch elm disease, chestnut blight, white pine blister rust thus emerged in native woody ecosystems, where they have caused irreparable damage. While these classic examples of tree diseases are well-known amongst mycologists and pathologists, it is important to realize that the emergence of similar new diseases has continued virtually unabated. New fungal pathogens have not only been introduced into new native forest ecosystems but pathogens of non-native plantation trees have been moved widely. In the latter case, the impacts have been variable and often times, it has been possible to manage disease problems through sylvicultural practices and tree breeding. This is, however, substantially depleting the profitability of intensive plantation forestry and in some cases businesses based on this practice are failing. To complicate matters, examples of fungal pathogens undergoing anthropogenic host-jumps are emerging. World-wide trends are clearly focused on attempts to stem the flow of tree pathogens to new environments. While success is achieved in some situations, this is an enormously complex problem and it seems likely that many new fungal diseases will continue to threaten world forests and forest industries in the foreseeable future.
Emerging fungal diseases threaten world forests

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3. Library
4. Museum
5. Art Gallery
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7. Casino
8. Police
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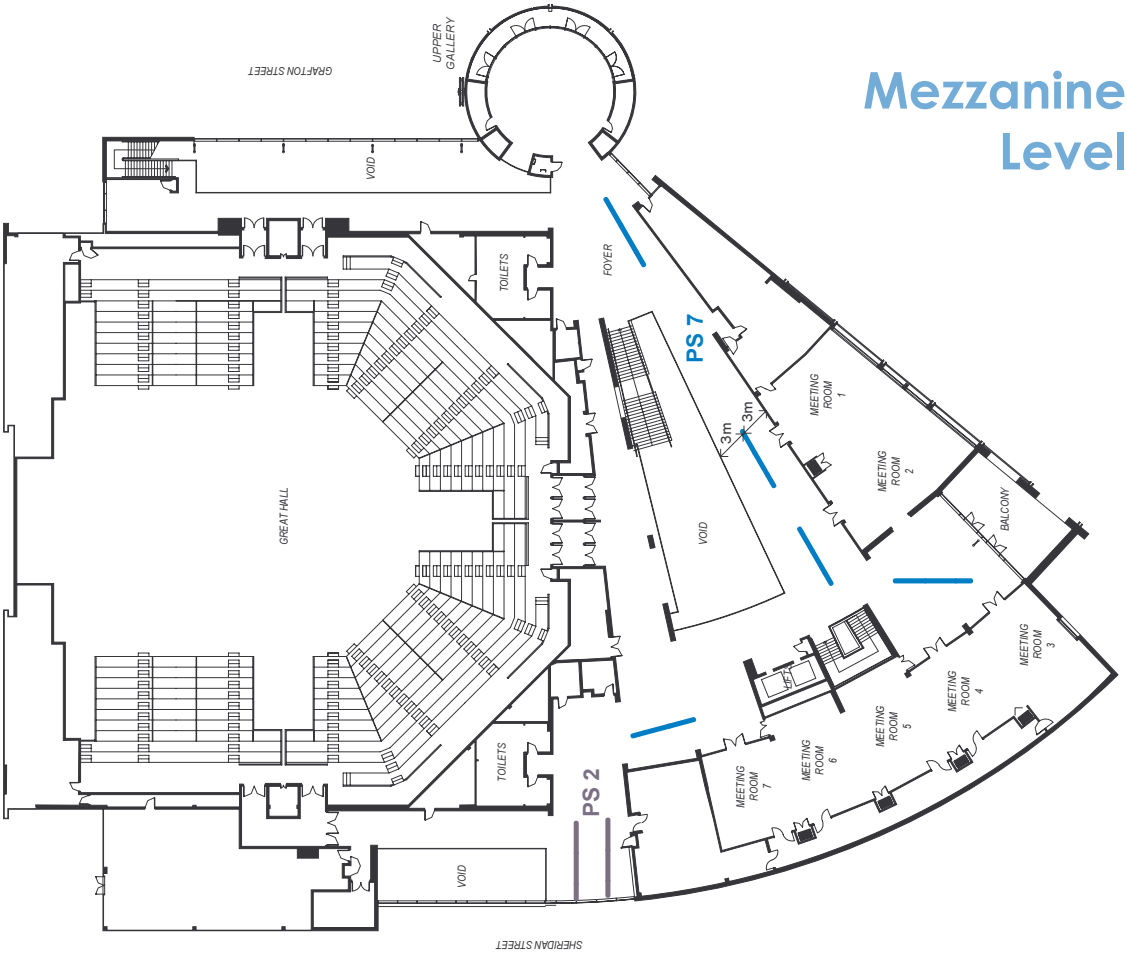
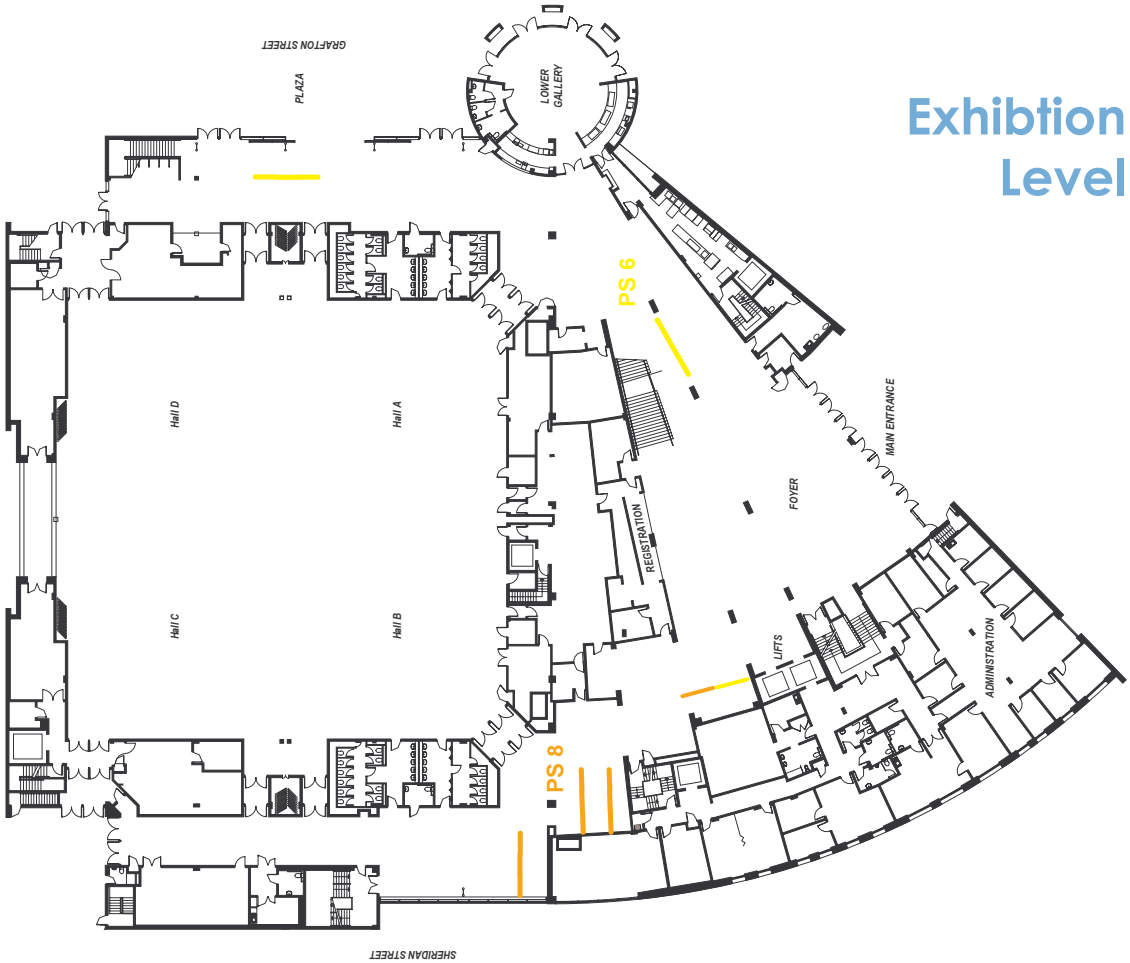
10. Cairns Convention Centre
11. National Bus Terminal
12. Ergon Energy Soundshell, Fogarty Park

- Information
- Bank
- Car park
- Hospital
- Toilets
- Railway Station

Mezzanine Level



Exhibtion Level



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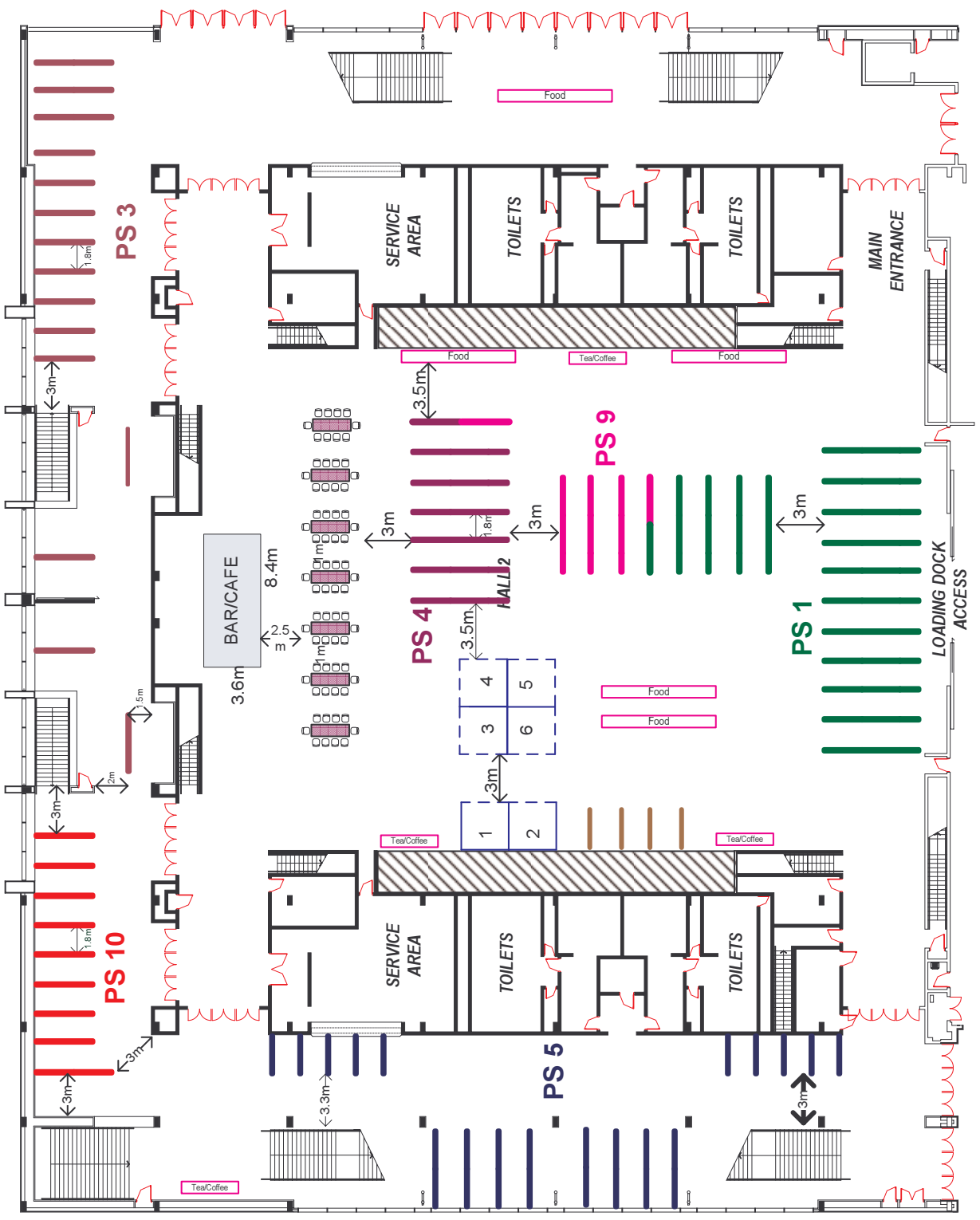

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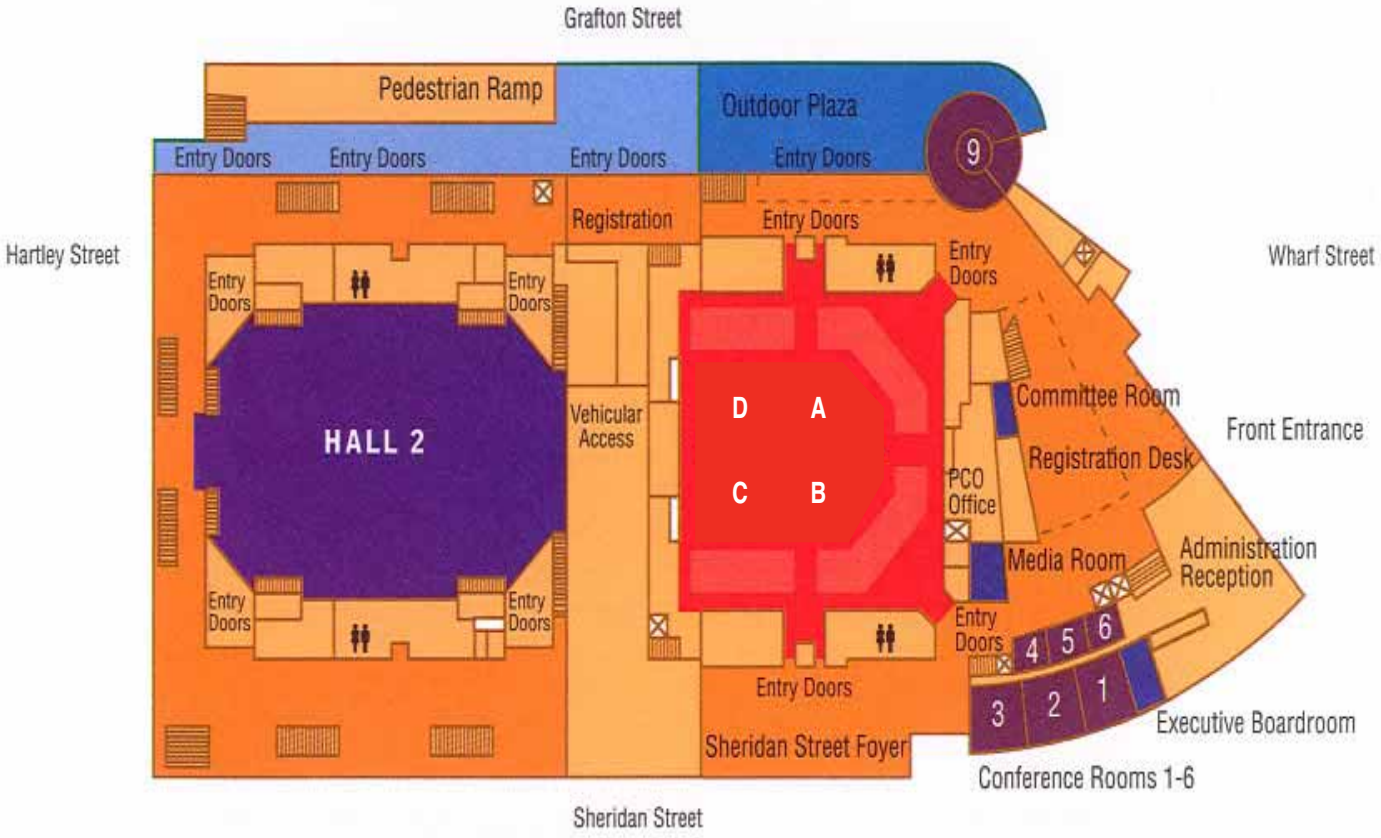

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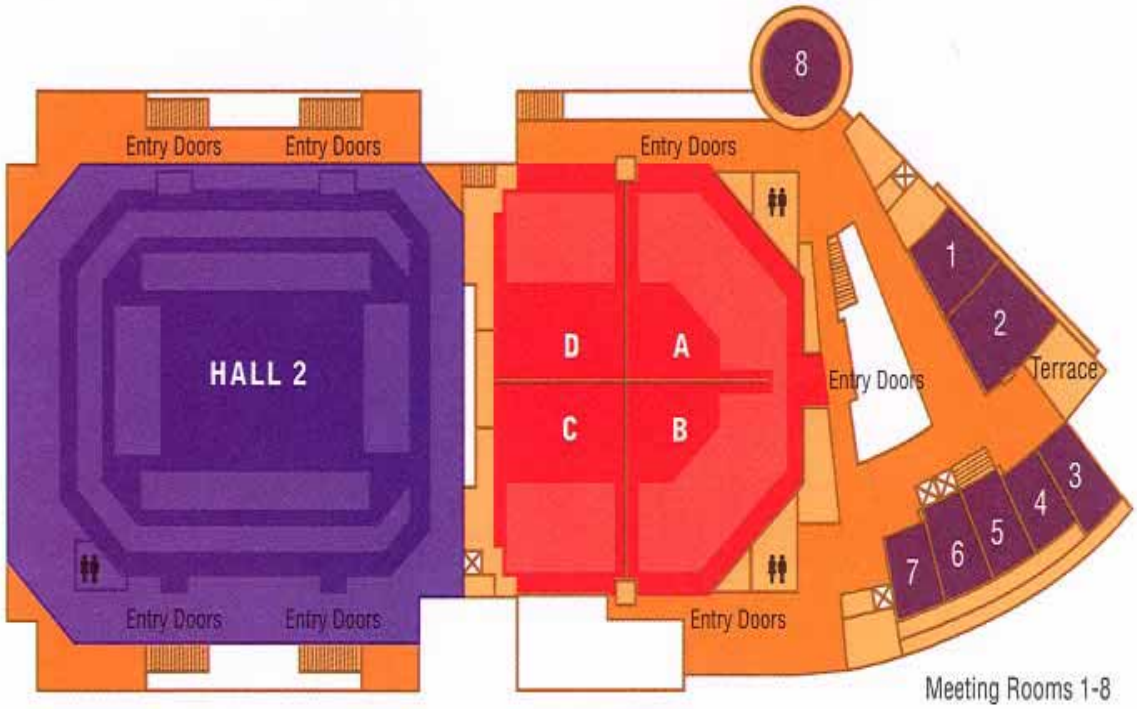


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