

IUBMB Focused Meeting on Extremophilic Fungi
Ljubljana, Slovenia, 19 – 22 September, 2023



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Abstract Book



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Edited by: Polona Zalar & Cene Gostinčar (University of Ljubljana, Biotechnical Faculty)
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Location & date: Ljubljana, Slovenia, 19-22 September, 2023
International Organising Committee: Nina Gunde - Cimerman (Slovenia), Cene Gostinčar (Slovenia), Ramón Batista (Mexico), Polona Zalar (Slovenia), Laura Selbmann (Italy)
Scientific Committee: Amy Gladfelter (USA), Jason Stajich (USA), Julia Schumacher (Germany), Michael Poulsen (Denmark), Vania A. Vicente (Brasil), Luiz Henrique Rosa (Brasil)
Local Organising Committee: Anja Černoša, Jerneja Čremožnik Zupančič, Klavdija Fortuna, Barbara Kastelic - Bokal, Monika Kos, Amela Kujović, Monika Novak Babič, Mojca Matul, Martina Turk
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Foreword

Nowadays we know that most environments that in the past were considered to be too extreme to be populated by any living form, can harbour life. Initially the prevailing conviction was that only prokaryotic cells, Bacteria and Archaea, are able to sustain the most extreme conditions and that eukaryotic cells, in particular fungi, are too complex to survive under such harsh conditions. In recent decades, however, fungi have been found to be an integral part of extreme microbial communities. Unlike their prokaryotic counterparts most fungi adapt to a wide range of an extreme condition, e.g. from no salt to saturation with salt, from low to high pH. They display a typical fungal strategy in adaptations – extremotolerance or even poly-extremotolerance - rather than extremophily. Extremotolerant fungi can be so adapted to multiple environments that they perceive a broad range of extreme conditions as optimal for their growth. Who are these fungi? Where can we find them? How did they evolve and how do they survive in some of the most extraordinary conditions we find on our planet? Which unique solutions to universal problems of adaptations they have evolved in comparison with prokaryotes? Can we use them in practical applications? Do they adapt to global climate change? Can they give us some astrobiological answers?

These are going to be the main questions that will be addressed at the first conference on extremophilic fungi that will bring together many world-renowned experts, young scientists, and students. Hopefully this first-time event will help usher mycology in the next era of extreme mycology and become a traditional event in the years to come.

Nina Gunde – Cimerman



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Mojca Matul
Martina Turk

Sessions:

- 1. Rock-Inhabiting Fungi**
- 2. Fungi in low water activity environments**
- 3. Fungi from Polar and Alpine Environments**
- 4. Astrobiology and Fungal Dispersal**
- 5. Interactions**
- 6. Radiation, Acidity and Other Extreme Environments I, II**
- 7. Black Fungi**
- 8. Omics and Molecular Tools for Extremophilic Fungi**
- 9. Biotechnology and Bioremediation**

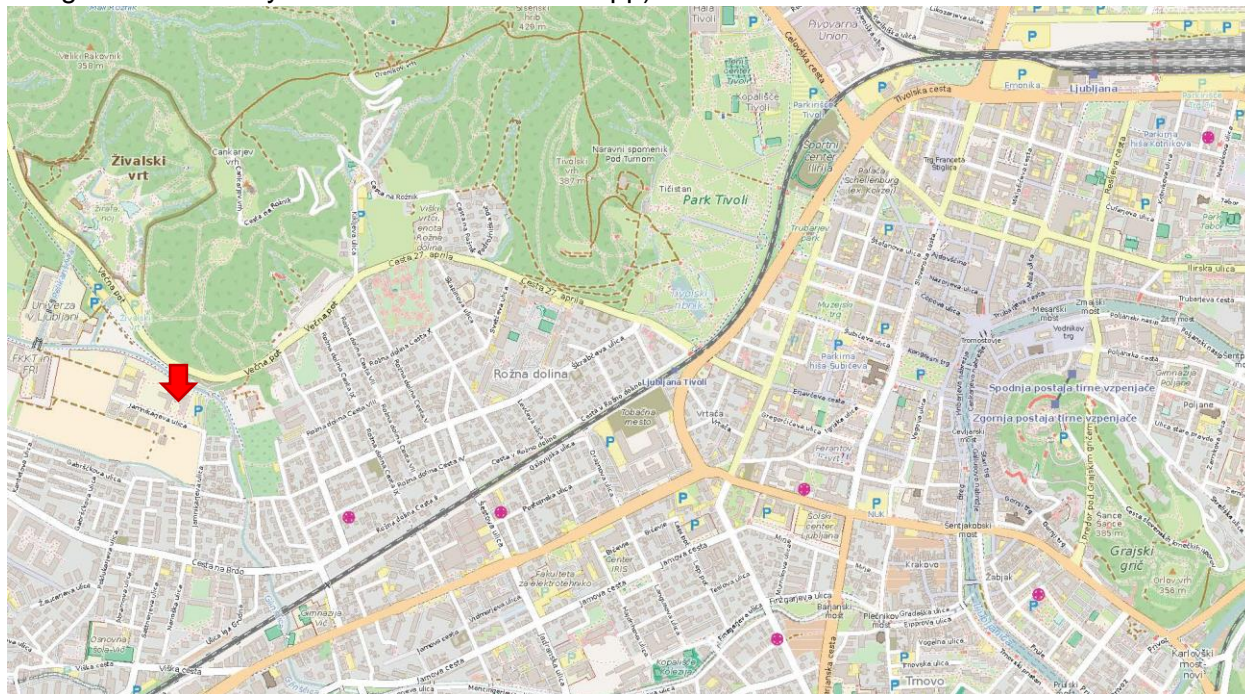
The text in the Abstract Book has not been modified by the editors. The authors take full responsibility for the submitted material. In exceptional cases, we have removed citations or acknowledgements because they exceeded the maximum length specified in the Guidelines for Authors.

General Information

Location

Biotechnical Faculty – University of Ljubljana, Jamnikarjeva 101, Ljubljana

The location can be reached from the city centre on foot (approximately 40 minute walk) or by public transport (bus lines 14 (bus stop JAMNIKARJEVA) and 18 or 18L (bus stop VEČNA POT) using an Urbana City Card or Urbana mobile app).



Internet Access

Wireless Internet Access is freely available in the faculty building.

Time Zone

Central European Time.

Climate

Moderate continental. Average daily temperatures in September range between 11 °C and 22 °C. September generally provides plenty of sunny days and possible occasional showers. Plan for the possibility of rain and chilly nights. For the scientific excursion please consider bringing good shoes appropriate for moderate hiking, warmer clothes and a waterproof layer for the cave visit (average temperature 12 °C with water dripping from the cave ceiling depending on precipitation) as well as adequate sun protection for the salterns visit.

Currency

Euro. ATMs are numerous, especially in city centres (extra charges may apply for withdrawing cash with foreign cards). Major credit cards are widely accepted, with few exceptions.

Information for Presenters

Lecture Guidelines

A computer with MS PowerPoint and a projector will be provided. A dedicated person will be present during breaks to help with uploading your presentations. The lectures shall be uploaded at the latest in the morning on the day of the presentation. Using alternative computers (e.g. personal laptops) for presentation will not be possible.

Available software: MS Office 2019 on Windows

Projection format: 16:9

Poster Guidelines

Posters should be in a portrait format and should not exceed the size A0 (width 841 mm × height 1189 mm / 33.1 × 46.8 inches). Material required to attach the posters will be provided.

Flashtalk Guidelines

Prepare a single PowerPoint slide in a 16:9 format and submit by e-mail prior to the conference.

Poster Awards

Microbiology Research, MDPI, will award a cash prize to the three best posters.

Other Information

Name Badge Policy

Participants (including accompanying persons) are kindly requested to display their conference name badges during all scientific and social events. Drinks at social events will be provided based on tokens received at registration.

Accompanying Persons

Accompanying persons are welcome to participate in the welcome reception, the full day scientific excursion to karstic caves and salterns and the conference dinner.

FEMS Early Career Scientist Meeting Grant Awardees

Marianna DOUROU (Department of Environmental Science and Policy, University of Milan, Milan, Italy)

Mija FRANKO (University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia)

Miha GLAVINA (University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia)

Alan Omar GRANADOS-CASAS (Unidad de Microbiología, Facultad de Medicina y Ciencias de la Salud, Universidad Rovira i Virgili, Reus, Spain)

Rafia Ahmed TULI (Institut des Substances et Organismes de la Mer, Nantes Université, Nantes, France)



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Biotehniška fakulteta



**Federation of European
Microbiological Societies**



**Frontiers in
Fungal Biology**



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Ljubljana Tourism



PROGRAMME

Tuesday, 19 September

9:00 – 9:30 Opening of the Conference

9:30 – 10:15 **Key Note Lecture:** Amy Gladfelter: Temperature adaptation of biological phase separation enables extremophilic lifestyles

1. ROCK-INHABITING FUNGI (10:15 – 11:00)

Session chairs: Lucia Muggia, Ramon Alberto Batista-García

10:15 – 10:45 Laura Selbmann: Rock Fungi: wired bodies, stunning potential

10:45 – 11:00 Martin Grube: Lichen partnerships: some notes about the most tolerant co-workers on earth

11:00 – 11:30 *Coffee Break*

2. FUNGI IN LOW WATER ACTIVITY ENVIRONMENTS (11:30 – 13:15)

Session chairs: Lucia Muggia, Ramon Alberto Batista-García

11:30 – 12:00 Ramon Alberto Batista-García: Cellular responses of *Aspergillus sydowii* to kosmotropic growth at extreme concentration of NaCl

12:00 – 12:30 Michael Poulsen: Adaptations to life in deserts and with termites in the globally-distributed basidiomycete genus *Podaxis*

12:30 – 13:00 Samah Mohamed Rizk Soliman: Exploring the surprising biodiversity: Extremophilic black fungal species from archaeological microhabitats in desert ecosystems

13:00 – 13:15 Bonnie K. Baxter: The Fungi of Great Salt Lake (Utah, USA): A survey of brine, minerals, petroleum, microbialites, and invertebrates

13:15 – 14:30 *Lunch and Poster Viewing*

3. FUNGI FROM POLAR AND ALPINE ENVIRONMENTS (14:30 – 16:00)

Session chairs: Laura Selbmann, Steven Hanes

14:30 – 15:00 Teppo Rämä: Intertidal fungi in the Arctic

15:00 – 15:30 Steven Hanes: Phase separation as a mechanism for cold tolerance

15:30 – 15:45 Ciro Sannino: Deep Antarctic Permafrost Core: relationships between the abundance of the yeast genus *Glaciozyma* and elemental composition

15:45 – 16:00 Lucia Muggia: Diversity of extremophilic fungi in highly heterogeneous lichen mycobiome

16:00 – 16:30 *Coffee Break*

4. ASTROBIOLOGY AND FUNGAL DISPERSAL (16:30 – 17:45)

Session chairs: Bonnie K. Baxter, Jason E. Stajich

16:30 – 17:00 Kathleen C. Benison: How can fungi in extreme acid lakes and their salts inform us about possible life on Mars?

17:00 – 17:30 Chris Koon Ho Wong: Fungal conidia prepares for the future according to environmental conditions present before dormancy

17:30 – 17:45 Marta Filipa Simões: Mycogenic metal nanoparticles for *in situ* resource utilization in microgravity environments

18:00 *Welcome Party*

Wednesday, 20 September

5. INTERACTIONS (9:00 – 9:30)

Session chairs: Vania A. Vicente, Teppo Rämä

9:00 – 9:15 *Victoria Keller*: Genomes of 14 black fungal lineages isolated from rock-dwelling lichens - a resource for understanding fungal lifestyles and environmental adaptations

9:15 – 9:30 *Danaé Bregnard*: For better or for worse: co-habitation of the fungus *Penicillium citrinum* and the yeast *Meyerozyma sp.* in deep geothermal fluids

9:30 – 10:30 POSTER PRESENTATIONS AND FLASH TALKS – PART 1

10:30 – 11:00 *Coffee Break*

6. RADIATION, ACIDITY AND OTHER EXTREME ENVIRONMENTS (11:00 – 13:00)

Session chairs: Elisabet Aranda, Chris Koon Ho Wong

11:00 – 11:15 *Michael J. Daly*: The role of Mn antioxidants in extremotolerant fungi

11:15 – 11:30 *Cene Gostinčar*: On the dispersal of fungi between extreme environments

11:30 – 12:00 *Janet Quinn*: Antimicrobial warfare: bacterial-mediated fungal killing through the Type VI secretion system

12:00 – 12:15 *Jens C. Frisvad*: The fungal production of specialized metabolites and proteins is depending on ecological strategy

12:15 – 12:30 *Anna A. Gorbushina*: Black fungi on technosphere surfaces: new niches for roof-inhabiting cousins

12:30 – 12:45 *Anna Poli*: The cultivable mycobiota of two karstic caves in Italy: undiscovered taxa and potential biotech applications

12:45 – 13:00 *Lenne Lange*: Evolution, composition, and biomass degrading capacity of the digestive secretome of 14 halophilic fungi

13:00 – 14:30 *Lunch and Poster Viewing*

6. RADIATION, ACIDITY AND OTHER EXTREME ENVIRONMENTS (cont.) (14:30 – 17:00)

Session chairs: Anna A. Gorbushina, Jens C. Frisvad

14:30 – 15:00 *Gustavo Goldman*: The extreme of the non-extremophiles: *Aspergillus fumigatus* drug tolerance and persistence

15:00 – 15:30 *Igor V. Grigoriev*: Comparative genomics of thermophilic fungi

15:30 – 15:45 *José Manuel Martínez*: Fungal diversity and its geochemical role in the extreme acidic environment of Río Tinto

15:45 – 16:00 *Iris Cornet*: Battle of extremophile *Rhodotorula kratochvilovae* strains for best process metrics in simultaneous detoxification and lipid production from steam explosion hydrolysate

16:00 – 16:30 *Coffee Break*

16:30 – 16:45 *Till Tiso*: Exploiting the black yeast *Aureobasidium pullulans* for polyamic acid production

16:45 – 17:00 *Ulrich Terpitz*: Green-light sensors in extremophilic fungi

17:00 – 18:00 POSTER PRESENTATIONS AND FLASH TALKS – PART 2

20:00 *Optional Social Gathering*

Thursday, 21 September

SCIENTIFIC EXCURSION AND WORKSHOP

Departure from the conference location: 8:00

Return to Ljubljana city centre or conference location: around 20:00

Please consider bringing:

- non-slippery walking shoes
- warm clothes (cave temperature approx. 12 °C, small amount of water dripping from the ceiling)
- sun protection
- enough drinking water
- additional food (a lunch box will be provided)

Morning

Scientific Excursion to Škocjan Caves

Afternoon

Workshop: Excursion and Potential Sampling of Hypersaline Environment in Sečovlje Man-Made Solar Salterns



<https://www.park-skocjanske-jame.si/en>



<https://www.soline.si/en>



Friday, 22 September

7. BLACK FUNGI (9:00 – 11:00)

Session chairs: Julia Schumacher, Macit Ilkit

9:00 – 9:20 Sybre de Hoog: Evolution of opportunism in Chaetothyriales

9:20 – 9:40 Macit Ilkit: Cerebral phaeohyphomycosis

9:40 – 10:00 Vania A. Vicente: Omics analysis of *Cladophialophora exuberance* focusing its potential on bioremediation of hydrocarbon and heavy metal polluted habitats

10:00 – 10:15 Erin C. Carr: Melanin excretion from the novel black yeast *Exophiala viscosa*

10:15 – 10:30 Ilaria Catanzaro: Generation of *Cryomyces antarcticus* mutants to explore the importance of DHN melanin for survival

10:30 – 10:45 Nadine J. Schneckenleitner: Contribution of DHN melanin to survival and growth of *Neoarthrinium moseri* in extreme environments

10:45 – 11:00 Eileen A. Erdmann: In-vivo mutagenesis of the rock inhabitant *Knufia petricola* by a customized Ac/Ds transposon system

11:00 – 11:30 *Coffee Break*

8. OMICS AND MOLECULAR TOOLS FOR EXTREMOPHILIC FUNGI (11:30 – 12:45)

Session chairs: Igor V. Grigoriev, Claudia Coleine

11:30 – 12:00 Jason E. Stajich: Genomic and phenotypic variation in *Rhodotorula* species sampled from extreme environments

12:00 – 12:30 Julia Schumacher: Genetic engineering of black fungi: Lessons learned from *Knufia petricola*

12:30 – 12:45 Lara Vimercati: The genome of the polyextremophilic yeast, *Naganishia friedmannii*, reveals adaptations involved in stress response pathways, carbohydrate metabolism expansion, and limited DNA repair repertoire

12:45 – 14:30 *Lunch and Poster Viewing*

9. BIOTECHNOLOGY AND BIOREMEDIATION (14:30 – 15:45)

Session chairs: Lene Lange, Gustavo Goldman

14:30 – 15:00 Nancy P. Keller: Isocyanides as tools of survival: Unveiling fungal strategies for copper starvation

15:00 – 15:30 Elisabet Aranda: Applications of isolated fungi for bioremediation purposes

15:30 – 15:45 Marianna Dourou: Non-conventional yeast: Investigating their potential for treatment of fish farm effluents and agro-industrial wastewaters

15:45 – 16:15 *Coffee break*

16:15 – 17:30 **Key Note Lecture and Closing of the Conference:** Nina Gunde-Cimerman: Water, water everywhere, nor any drop to drink

19:30 *Conference Dinner*

FUN-EX List of presentations



Ljubljana, September 19th-22nd 2023

KEYNOTE= key note lecture; IL=invited lecturer / S=speaker / P=poster

KEYNOTE LECTURE			
Authors	Title	Presenta- tion No.	Page No.
Ben Stormo, <u>Amy S Gladfelter</u>	Temperature adaptation of biological phase separation enables extremophilic lifestyles	Keynote, IL1	26

Session 1 Rock-Inhabiting Fungi			
Authors	Title	Presenta- tion No.	Page No.
LECTURES			
<u>Laura Selbmann</u>	Rock Fungi: wired bodies, stunning potential	IL2	28
<u>Martin Grube</u>	Lichen partnerships: some notes about the most tolerant coworkers on earth	S1.1	29
POSTERS			
<u>Claudio G. Ametrano</u> , Laura Selbmann, Lucia Muggia	STRES project delivers eleven new draft genomes from never sequenced genera of melanized Eurotiomycetes and Dothideomycetes isolated from lichens	P1.1	81
<u>Domenico Celi</u> , Letizia Berti, Massimiliano Marvasi, Brunella Perito	Characterization of the community of Rock Inhabiting Fungi associated to white marble of Florence Cathedral	P1.2	82

Session 2 Fungi in low water activity environments			
LECTURES			
Authors	Title	Presenta- tion No.	Page No.
<u>Ramón Alberto Batista-García</u>	Cellular responses of <i>Aspergillus sydowii</i> to kosmotropic growth at extreme concentration of NaCl	IL3	31
<u>Michael Poulsen</u>	Adaptations to life in deserts and with termites in the globally-distributed basidiomycete genus <i>Podaxis</i>	IL4	32
<u>Samah Mohamed Rizk, Mahmoud Magdy</u>	Exploring the Surprising Biodiversity: Extremophilic Black Fungal Species from Archaeological Microhabitats in Desert Ecosystems	IL5	33
<u>Bonnie K. Baxter, Carly Biedul, Cayla Martin, Paulina Martinez-Koury, and Cora Rasmuson, Mary Sanchez</u>	The Fungi of Great Salt Lake (Utah, USA): A survey of brine, minerals, petroleum, microbialites, and invertebrates	S2.1	34
POSTERS			
<u>Domenico Davolos, Alessandra Ricelli, Oriana Maggi, Andrea Ceci, Persiani Anna Maria, Valeria Prigione, Francesco Venice, Giovanna Cristina Varese</u>	Draft genome annotation of the oosporein-producing <i>Victoriomyces antarcticus</i> strains MUT3686, MUT5898 and MUT5937 (Sordariomycetes, Cephalothecaceae) isolated from Antarctic soils and African desert oases	P2.1	84
<u>Miha Glavina, Kathleen C. Benison, Polona Zalar, Nina Gunde-Cimerman</u>	Dormancy of extremotolerant <i>Parengyodontium torokii</i> in salt crystals	P2.2	85
<u>Alan Omar Granados-Casas, María Barnés Guirado, Ana Fernández-Bravo, Alberto Miguel Stchigel, José Francisco Cano-Lira</u>	Comparative study of the fungal biota of a hypersaline and a freshwater lagoon in Spain	P2.3	86
<u>José M. Martínez, Andrea Irene Silva-Claros, Borja Rodríguez de Francisco, Kary Haro, Adrián Martínez Bonilla, Nuria Rodríguez, Ricardo Amils</u>	New species of chaophilic fungus isolated in hypersaline sediments of Salar de Uyuni	P2.4	87
<u>David L Parrott, Jr, Haley K Nate</u>	Shoreline Soil Fungal Microbiomes at Great Salt Lake, Utah	P2.5	88
<u>María del Rayo Sánchez Carbente, Yordanis Pérez Llano, Ramón A. Batista García, Víctor Manuel Ocampo Medina, Eya Caridad Rodríguez Pupo, Nina Gunde Cimerman, Rok Kostanjšek, Jorge Luis Folch Mallol</u>	Lignocellulosic degradation on hypersaline conditions by moderate halophile <i>A. sydowii</i>	P2.6	89

<u>Weronika Śliżewska</u> , Katarzyna Struszczyk-Świta, Joanna Oracz, Anna Otlewska, Olga Marchut-Mikołajczyk	Pigments from halophilic filamentous fungi isolated from saline soils	P2.7	90
Rafia <u>Ahmed Tuli</u> , Elise Gerometta, Bastien Cochereau, Emmanuel Gentil, Samuel Bertrand, Laurence Meslet-Cladière, Deniz Tasdemir, Nina Gunde-Cimerman, Monika Kos, Catherine Roullier	Metabolomic study of a halotolerant black yeast: <i>Hortaea werneckii</i>	P2.8	91
<u>Iñigo Zabalgogezcoa</u> , Eric C. Pereira, Beatriz R. Vázquez de Aldana	Structure and functions of the fungal microbiome of <i>Festuca pruinosa</i> , a chasmophytic and halophytic grass	P2.9	92
<u>Polona Zalar</u> , Amela Kujović, Martina Turk, Cene Gostinčar, Mojca Matul, Vanja Miklavčič, Veronika Mrak, Katja Kavkler, and Nina Gunde-Cimerman	Xerophilic fungi contaminate cultural heritage objects on display indoors	P2.10	93
<u>Arianna Petrucci</u> , Luen Zidar, Černoša, Anja, Cene Gostinčar, Nina Gunde-Cimerman	Desiccation induced extremotolerance: a preliminary study	P2.11	94
Benito Gómez-Silva, Nicomedes Valenzuela-Lopez	Primary evidence of new species for <i>Penicillium</i> Section <i>Chrysogena</i> colonizing halites at Salar Grande, Atacama Desert	P2.12	95

Session 3 Fungi from Polar and Alpine Environments			
Authors	Title	Presenta- tion No.	Page No.
LECTURES			
<u>Teppo Rämä</u>	Intertidal fungi in the Arctic	IL6	36
Nathan McKean, Claudia Coleine, Laura Selbmann, Cene Gostinčar, Nina Gunde-Cimerman, Alaji Bah, <u>Steven D. Hanes</u>	Phase separation as a mechanism for cold tolerance	IL7	37
<u>Ciro Sannino</u> , Gianmarco Mugnai, Luigimaria Borruso, Alessandro Bernetti, Daniele Andreani, Pietro Buzzini, Benedetta Turchetti, Mauro Guglielmin	Deep Antarctic Permafrost Core: relationships between the abundance of the yeast genus <i>Glaciozyma</i> and elemental composition	S3.1	38
Claudio G. Ametrano, Agnese Cometto, Steven D. Leavitt, Martin Grube, Alberto Pallavicini, Sybren de Hoog, <u>Lucia Muggia</u>	Diversity of extremophilic fungi in highly heterogeneous lichen mycobiome	S3.2	39

POSTERS			
<u>Claudia Coleine</u> , Davide Albanese, Cassandra Ettinger, Manuel Delgado-Baquerizo, Claudio Donati, Christa Pennacchio, Susannah Tringe, Jason E. Stajich, Laura Selbmann	Metagenomes untangle diversity and adaptation of Antarctic lichen-dominated endolithic communities	P3.1	97
<u>Jerneja Čremožnik Zupančič</u> , Mojca Matul, Polona Zalar and Nina Gunde-Cimerman	Fungi from polar and alpine environments also colonise household refrigerators	P3.2	98
<u>Monika Laichmanová</u>	ITS sequence analysis of rock-inhabiting fungi isolated from James Ross Island, Antarctica	P3.3	99
<u>Laura Perini</u> , Thomas Turpin-Jelfs, Athanasios Zervas, Katie Sipes, Carsten S. Jacobsen, Liane G. Benning, Martyn Tranter, Alexandre M. Anesio	Chytridiomycetous fungi are major controls of glacier ice algae biomass on the Greenland Ice Sheet	P3.4	100

Session 4 Astrobiology and Fungal Dispersal			
Authors	Title	Presenta- tion No.	Page No.
LECTURES			
<u>Kathleen C. Benison</u> , John E. Hallsworth, Polona Zalar, Miha Glavina, Martha E. Gibson, Karena K. Gill, Nicolas Randazzo, Nina Gunde-Cimerman	How can fungi in extreme acid lakes and their salts inform us about possible life on Mars?	IL8	41
<u>Chris Koon Ho Wong</u>	Fungal conidia prepares for the future according to environmental conditions present before dormancy	IL9	42
<u>Marta Filipa Simões</u> , Allen Drews, Donatella Tesei, Junjie Zhang, Cristiane Ottoni, André Antunes	Mycogenic metal nanoparticles for in situ resource utilization in microgravity environments	S4.1	43
POSTERS			
Allen Matt Drews, Lei Jin, Marta Filipa Simões	Exploring filamentous fungi biofilms under simulated microgravity on analog Martian and Lunar regoliths	P4.1	102
Donatella Tesei, Marta Filipa Simões	Screening of extremotolerant filamentous fungi for the biogenic synthesis of metal nanoparticles under simulated microgravity	P4.2	103

Session 5 Interactions			
Authors	Title	Presentation No.	Page No.
LECTURES			
<u>Victoria Keller</u> , Anjuli Calchera, Jürgen Otte, Imke Schmitt	Genomes of 14 black fungal lineages isolated from rock-dwelling lichens – a resource for understanding fungal lifestyles and environmental adaptations	S5.1	45
<u>Danaé Bregnard</u> , Saskia Bindschedler, Simona Regenspurg, Pilar Junier	For better or for worse: co-habitation of the fungus <i>Penicillium citrinum</i> and the yeast <i>Meyerozyma</i> sp. in deep geothermal fluids	S5.2	46
POSTERS			
<u>De Carolis Roberto</u> , Gerardo Antonio Stoppiello, Claudia Coleine, Laura Selbmann, Lucia Muggia	Lichen mycodiversity in Antarctica reveal the key role of thallus structure as suitable niche for diversity	P5.1	105
<u>Imke Schmitt</u> , Victoria Keller, Jürgen Otte	A lichen-associated black fungus distributed across continents, climate zones, and host species	P5.2	106

Session 6 Radiation, Acidity and Other Extreme Environments			
Authors	Title	Presentation No.	Page No.
LECTURES			
<u>Michael J. Daly</u>	The Role of Mn Antioxidants in Extremotolerant Fungi	IL10	48
<u>Cene Gostinčar</u> , Anja Černoša, Martina Turk, Polona Zalar, Nina Gunde-Cimerman	On the dispersal of fungi between extreme environments	S6.1	49
<u>Janet Quinn</u> , Katharina Trunk, Yasmin Ahmed, Maisie Palmer, Genady Pankovs, William Hunter, Sarah Coulthurst	Antimicrobial warfare: bacterial-mediated fungal killing through the Type VI secretion system	IL11	50
<u>Jens C. Frisvad</u>	The Fungal production of specialized metabolites and proteins is depending on ecological strategy	S6.2	51
<u>Anna A. Gorbushina</u> , Julia Schumacher, Khaled Abdallah	Black fungi on technosphere surfaces: new niches for roof-inhabiting cousins	S6.3	52

<u>Anna Poli</u> , Andrea Zanellati, Federico Biagioli, Claudia Coleine, Marco Isaia, Elena Piano, Laura Selbmann, Cristina Varese, Valeria Prigione	The cultivable mycobiota of two karstic caves in Italy: undiscovered taxa and potential biotech applications	S6.4	53
<u>Lene Lange</u>	Evolution, composition, and biomass degrading capacity of the digestive secretome of 14 halophilic fungi	S6.5	54
<u>Gustavo H. Goldman</u>	The extreme of the non-extremophiles: <i>Aspergillus fumigatus</i> drug tolerance and persistence	IL12	55
Andrei S. Steindorff, Maria Victoria Aguilar Pontes, Donald O. Natvig, Amy J. Powell, Adrian Tsang, <u>Igor V. Grigoriev</u>	Comparative Genomics of Thermophilic Fungi	IL13	56
<u>José Manuel Martínez</u> , Monike Oggerin, Catalina del Moral, Nuria Rodríguez, Nuria Fernández, Ricardo Amils	Fungal diversity and its geochemical role in the extreme acidic environment of Río Tinto	S6.6	57
<u>Iris Cornet</u> , Waut Broos, Nina Gunde-Cimerman	Battle of extremophile <i>Rhodotorula kratochvilovae</i> strains for best process metrics in simultaneous detoxification and lipid production from steam explosion hydrolysate	S6.7	58
<u>Till Tiso</u> , Difan Xiao, Lars M. Blank	Exploiting the black yeast <i>Aureobasidium pullulans</i> for polymalic acid production	S6.8	59
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PRESENTATIONS

Temperature adaptation of biological phase separation enables extremophilic lifestyles

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[1] Duke University, Durham, NC, USA

Free-living microbes, plants and cold-blooded organisms survive in the face of environmental fluctuations that arise across many time scales. Climate change is increasing the amplitude and frequency of environmental variations in the natural world and biological phase separation may be a key mechanism of adaptation of the biosphere to climate change. The focus of this presentation will be on our recent work to identify how protein and RNA sequence encodes temperature sensitivity and how material properties of biomolecular condensates are maintained across temperatures fluctuations. For these studies, we focus on a model phase separation protein, Whi3, in the syncytial ascomycete fungus, *Ashbya gossypii*. This protein binds to and regulates specific RNAs important for cell cycle control and cell polarity. We have found natural sequence variation within the core protein/RNA components are sufficient to induce highly tunable temperature sensitivity for condensation. Sequence elements controlling protein-protein, protein-RNA and RNA-RNA interactions all contribute to modulating higher-order assembly and function in different temperature regimes. These studies indicate that small changes in protein and RNA sequences can promote organism adaptation to different climates providing potential mechanisms for adaptation of the biosphere to climate change. Discussion of how the physical organization of cytosol via biomolecular condensates impacts the adaptability of extremophile fungi will be a goal of the presentation.

Session 1 - Rock-Inhabiting Fungi

Rock Fungi: wired bodies, stunning potential

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Rock is a very unattractive niche colonized by specialists only, able to tolerate the wide plethora of stresses insisting on exposed surfaces including intense solar radiation, extensive oligotrophy, prolonged water depletion and temperature fluctuations. Rock Inhabiting Fungi (RIF) are a striking example of obligate rock dwellers. Discovered in hot and cold deserts, RIF occur from Antarctica to the Mediterranean, on natural outcrops and monuments. They were overlooked for a long time due the cultivation difficulties for their very slow growth and poor competitive abilities or just ignored because considered a nuisance or irrelevant. The refinement of targeted culturing techniques and rapid progresses of sequencing technologies revealed their polyphyletic origin and astounding biodiversity, contrasting with shared morpho-physiology and poor differentiation; it became evident not only the splendid opportunities of research in many different basic and applied fields of “rock dark matter”, but also to approach questions of wide breath as untangle adaptation mechanisms in the extremes, understand the possibilities for life in a drying world or even beyond earth.

Multiple-stress exposition shaped their extraordinary ability to cope with physical stresses such as extreme pH and temperature and desiccation with tremendous capacity to resurrect. They also survive UV and ionizing radiation well beyond lethal dose for the most. Recent studies on 118 RIF and lithotolerant fungi unearthed that surviving ionizing radiation was related both to taxonomy and pressure characterizing the environment where they live. Digging into their genomes, radioresistance resulted associated to the capacity in DNA repair and protection against reactive oxygen species. Larger genomes with higher number of genes and CG content were associated with harshest conditions and include potential diploid and triploid lineages. A further contribution to our acquaintance with RIF is expected from the “Shed Light in the daRk lineagES of the Fungal tree of life” (STRES) project.

Lichen partnerships: some notes about the most tolerant coworkers on earth

Martin Grube

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Lichens are commonly cited for their excessive tolerance to adverse conditions, be it the extremes of special terrestrial habitats or human tortures such as acetone rinsing or exposure to outer space conditions. As poikilohydric organisms, they survive extremes usually in dry state, whereas they are usually more sensitive in metabolically active state. The latter explains the diversification and specialization of lichens in their ecological niches, ranging from salt-sprayed coasts, to cold and hot deserts and to the summits of high mountains, where the partnerships are exposed to extreme dynamics of temperatures, high radiation and excessive drought. Beyond their tolerance to drought, certain lichens cope with excessive levels of metals, which they detoxify and accumulate in their thalli. In this contribution I present the current knowledge about extremotolerance in lichens and the underlying mechanisms of the partnering organisms, which seem to mutually support survival under extreme conditions.

Session 2 - Fungi in Low Water Activity Environments

Cellular responses of *Aspergillus sydowii* to kosmotropic growth at extreme concentration of NaCl

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Although various studies have investigated osmoadaptations of halophilic fungi to saline conditions, only few analyzed the fungal mechanisms occurring at saturated concentrations (NaCl, KCl). Halophilic *Aspergillus sydowii* is a model organism for the study of molecular adaptations of filamentous fungi to hyperosmolarity. For the first time a multi-omics approach (i.e., transcriptomics and metabolomics) was used to compare *A. sydowii* at saturated concentration ($a_w=0.75$; NaCl and KCl) to optimal salinity ($a_w=0.99$; 0.5 M NaCl and KCl). Also, the analysis included the fungal growth in the presence of sorbitol at $a_w=0.75$ and $a_w=0.99$. Analysis revealed 1,842 genes differentially expressed of which 704 were overexpressed at $a_w=0.75$. Most differentially expressed genes were involved in metabolism and signal transduction. A gene ontology multi-scale network showed that ATP binding constituted the main network node with direct interactions to phosphorelay signal transduction, polysaccharide metabolism and transferase activity. Free amino acids significantly decreased and amino acid metabolism was reprogrammed at $a_w=0.75$. mRNA transcriptional analysis revealed upregulation of genes involved in methionine and cysteine biosynthesis at extreme water deprivation by NaCl. No modifications of membrane fatty acid composition occurred. Upregulated genes were involved in high-osmolarity glycerol signal transduction pathways, biosynthesis of β -1,3-glucans and cross membrane ion transporters. Downregulated genes were related to the synthesis of chitin, mannose, cell wall proteins, starvation, pheromone synthesis, and cell cycle. Non-coding RNAs represented the 20% of the total transcripts with 7% classified as long non-coding RNAs (lncRNAs). The 42% and 69% of the total lncRNAs and RNAs encoding transcription factors, respectively, were differentially expressed. A network analysis showed that differentially expressed lncRNAs and RNAs coding transcriptional factors were mainly related to the regulation of metabolic processes, protein phosphorylation, protein kinase activity, and plasma membrane composition. Metabolomic analyses revealed more complex and unknown metabolites at saturated NaCl concentration than at optimal salinity. This study is the first attempt to unravel the molecular ecology of an ascomycetous fungus at extreme water deprivation by NaCl, KCl and sorbitol. This work also represents a pioneer study to investigate the importance of lncRNAs and transcriptional factors in the transcriptomic response to high NaCl stress in halophilic fungi. Finally, we also employed solid-state NMR spectroscopy to compare the cell wall architecture of *Aspergillus sydowii* and other halophilic and halotolerant fungi across salinity gradients. Analyses of intact cells reveal that *A. sydowii* cell walls contain a rigid core comprising chitin, β -glucan, and chitosan, shielded by a surface shell composed of galactomannan and galactosaminogalactan.

Adaptations to life in deserts and with termites in the globally-distributed basidiomycete genus *Podaxis*

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The genus *Podaxis* is distributed across all continents except for the antarctica and either lives free-living (in Eurasia and the Americas) or in association with *Trinervitermes* (Africa) or *Nasutitermes* (Australia) termites. To uncover the global diversity of the genus, we obtained basidiospore samples and photographs from 165 herbarium specimens covering 33 countries. Combining phylogenomic analysis from 3,839 BUSCO genes derived from low-coverage genomes with morphology, ecology (free-living vs. termite-associated), and geographical distribution, we established that the genus includes at least 16-22 species. Capitalizing on being able to revive spores from centuries-old specimens, we further generated high-quality genomes of 10 specimens, including two type species described by Linnaeus >240 years ago. Comparative genomics revealed that termite association was accompanied by significant genome reduction, accelerated evolution in protein-coding genes, and reduced functional capacities for oxidative stress responses and lignin degradation. Functional testing confirmed that termite-associated species perform worse under oxidative stress, while all lineages retained some capacity to cleave lignin. Mitochondrial genomes of termite-associated species were significantly larger than free-living counterparts, possibly due to smaller population sizes or reduced competition. In conclusions, the rich collection of herbarium specimens of *Podaxis* enabled robust resolution of the diversity of the understudied genus and provided the first indications of adaptations and genomic consequences of the evolution of termite association.

Exploring the Surprising Biodiversity: Extremophilic Black Fungal Species from Archaeological Microhabitats in Desert Ecosystems

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There was a time when desert regions were believed to be too hostile for any form of life to thrive. With today's knowledge, deserts are full of life, representing all domains and all kingdoms. Without a doubt, deserts trigger a variety of adaptation mechanisms in microorganisms as they provide the most extreme conditions on earth. Among the main and most important stress factors in the desert are the constant or temporal matrix stress caused by the limited availability of water, the high temperature and sudden temperature changes, as well as the osmotic stress caused by salt accumulations in the soil or the rock surfaces. Microbial communities in low water environments contribute to essential ecological processes, such as nutrient cycling, organic matter decomposition, and mineral weathering. Their interactions with the rock substrates can lead to physical, chemical, and biological changes, affecting the stability and preservation of archaeological sites. Furthermore, their metabolic activities may have implications for the conservation of cultural heritage materials, as some microbes can contribute to the deterioration or preservation of archaeological artifacts and structures. In Egypt, a hyper-arid region, a vast study was conducted to isolate extremotolerant black fungi from the famous prehistoric monuments of Necropolis Egypt, namely: Djoser's Step Pyramid, The Great Pyramid of Giza King Khufu's Pyramid, and King Senusret II Lahun Pyramid. Based on metabarcoding analysis, multi-locus genotyping (ITS, nrSSU or 18S, nrLSU or 28S), and morphological data, new records of extremotolerant ecotypes from the black yeast *Hortaea werneckii*, and rock inhabiting fungi *Pseudotaeniolina globosa* and *Knufia karalitana*, were confirmed and reported. An extremophilic profile for the Egyptian isolates was observed with significant growth rates under various extreme environmental conditions, such as wider temperature range (4 - 37 °C), and pH values (3.0 - 9.0 pH), as well as extreme salinity levels (5 M NaCl).

The Fungi of Great Salt Lake (Utah, USA): A survey of brine, minerals, petroleum, microbialites, and invertebrates

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Microbial diversity studies at Great Salt Lake have focused primarily on bacteria, archaea, and microalgae and their significance in the ecosystem. The gap in the data on fungi may lead to misunderstandings about the lake's ecology. A recent study that focused on cultivation demonstrated a rich diversity of fungi that live in the hypersaline brine of the salt-saturated north arm. The fungal diversity in the less saline south arm is expectedly larger but has not been explored. Nor have some of the more interesting niches associated with the lake, including the interior of minerals, petroleum seeps, microbialites, and the invertebrates that live in the south arm. PCR amplification with internal transcribed spacer (ITS) gene primers of these genetic reservoirs revealed a variety of fungi. After the demonstration of methane emissions at the Rozel Point tar seeps, we sampled three locations where the asphalt seeps out of the ground around the margins of the Great Salt Lake north arm. Fungal species were identified that may degrade Polycyclic aromatic hydrocarbons (PAHs): *Cladosporium cladosporioides*, *Penicillium citrinum*, *Penicillium commune*, *Epicoccum nigrum*, and *Rhodotorula marina*. In addition, more than 14 distinct genera of fungi were identified from entombed microbial communities in gypsum crystals dug from the clays around the shoreline. In the gypsum interior, the genera present with the highest abundance were *Ophiocordyceps*, a parasitic insect fungus, and representatives from the commensal genus *Malassezia*. We are currently exploring the pupae of the brine fly, *Ephydra gracilis*, and the cysts of the brine shrimp, *Artemia franciscana*, for evidence of fungi in their microbiome. We will also present data on fungal components of the lake's microbialite mats, critical structures that support brine fly pupation. These results, taken together, will provide a more wholistic picture of the roles of fungi in the Great Salt Lake ecosystem.

Session 3 - Fungi from Polar and Alpine Environments

Intertidal fungi in the Arctic

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Intertidal zone with ebbing and flowing and high variation in temperature and other abiotic factors represents an extreme environment for organisms. This is especially the case in the Arctic where summertime water temperature is just some Celsius degrees and at the same time air temperatures can reach up to 36 C in the intertidal zone. In the winter, freezing and varying wind and rain conditions cause extra stress for marine intertidal organisms during low tides, whereas during high tides they need to cope with low water potential and dilution effect of the seawater. Also, ice erosion and wave action at exposed sites cause additional mechanical stress. Marine fungi in the intertidal show morphological, physiological, ecological and genomic adaptations that help them to survive and even flourish in this extreme environment forming diverse communities in different kinds of shores. Moreover, endophytic fungi contribute directly to the flourishing of macroscopic hosts. This talk presents recent research on the diversity and ecology of intertidal fungi and peculiar features helping them to thrive in this extreme environment.

Phase separation as a mechanism for cold tolerance

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We propose that macromolecular phase separation, a phenomenon long known to chemists and physicists, is used by microbial organisms to survive in extreme environments. Phase separation in biology is characterized by the process whereby intrinsically disordered regions (IDRs) of proteins engage in weak, multivalent interactions (often with nucleic acids) to separate from the bulk solvent forming biomolecular condensates. Some examples of biomolecular condensates include P-bodies, stress granules, the nucleolus, and so-called “transcription factories” formed by RNA polymerase II. Phase separation is reversible, and can act as a sensor for various cellular stresses. It can also create localized microenvironments separate from the surrounding cellular milieu that are optimized for specific biological processes. Based on these features and our preliminary studies, we are testing two hypotheses. *First*, that biophysical properties of IDRs, including phase separation, can be fine-tuned by evolutionary selection for function in extreme environments. *Second*, that phase separation provides a mechanism to concentrate enzymes with their substrates to overcome energetic barriers to metabolic activity in extreme cold environments. To test these hypotheses, we are studying cold-adapted fungi from the Arctic and Antarctic, as well as halophilic species capable of growth in cold, low water environments. We found that the RNA polymerase II carboxy terminal domain (CTD), which is an IDR that mediates phase separation *in vitro*, is highly divergent in these extremophilic fungi. This divergence endows the CTDs with unique phase separation properties *in vitro*, and impacts their behavior in functional tests *in vivo*. Our results are consistent with the idea that environmentally-tuned, evolutionarily selected phase separation by the CTD and IDRs in other proteins plays an adaptive role in tolerance to cold and potentially other extreme environments.

Deep Antarctic Permafrost Core: relationships between the abundance of the yeast genus *Glaciozyma* and elemental composition

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Fungal communities represent an important portion of cold-adapted Antarctic microbial diversity and play an essential role as nutrient cyclers and organic matter mineralizers. The knowledge of the chemical environmental parameters that putatively drive microbial abundance and diversity may be crucial for elucidating the ecology of fungal communities in Antarctic ecosystems.

In this framework, the chemical-physical parameters (i.e. TOC, TON, TOP, elemental composition, electrical conductivity, pH, and water content) and the structure of fungal communities (sequencing the ITS2 of the eukaryotic ribosomal operon using the NGS approach) of a six meters depth permafrost core have been studied. The permafrost core (drilled in a rock glacier located on the southern side of Adélie Cove site, Northern Victoria Land, Continental Antarctica) was divided into five units based on depth considering the different ice content. Alpha- and beta-diversity of fungal communities of the different units were obtained and the Pearson correlation coefficient was used for calculating co-occurrences among chemical-physical parameters and the dominant fungal genera (relative abundance > 2%).

Depth-related significant ($p < 0.05$) differences were found in chemical-physical parameters, and alpha- and beta-diversity of fungal communities. Yeast life forms dominated the fungal diversity of all the permafrost cores (Basidiomycota was the dominant phylum). Interestingly, a high abundance (about two-thirds of the total reads) of the yeast genus *Glaciozyma* was found in the deepest layer; this high abundance resulted correlated with the elemental composition of the core (especially with the concentration of Mg, Ca, S, K, and Sr). This result may be considered extremely rare in Antarctic yeast diversity. Possible chemical-physical and geological hypotheses supporting such dominance of the genus *Glaciozyma* are suggested.

Diversity of extremophilic fungi in highly heterogeneous lichen mycobiome

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Lichens are multi-kingdom symbioses in which fungi, algae and bacteria interact to develop a stable selection unit. Lichens are also known to be reservoirs of other fungi that choose the thalli as own ecological niche to thrive in more extreme habitats. Heterogeneous and largely transient fungal communities associated with lichens and representing the lichen mycobiome have been in part described, but their taxonomic and functional diversity across the range of their host lichens is still largely unknown. We characterized the diversity of the lichen mycobiomes in two cosmopolitan lichens, i.e. *Rhizoplaca melanophthalma* and *Tephromela atra* as study models, across their geographic distributional range worldwide (including dry, high altitude extreme environments), using both a culture-dependent approach and environmental DNA. We discovered that the variation of the mycobiomes associated with the two lichen species is extremely high and is mainly represented by extremophilic taxa. A stable species-specific core mycobiome is hardly identifiable, and most of the mycobiome taxa are present in low frequency of occurrence. Furthermore, a fraction of the two lichen mycobiomes is detectable only by the culture-dependent approach and escapes PCR amplification. Although there is no taxon ubiquitously present in neither lichen species, among the successfully isolated taxa, two of them have been confirmed to represent extremophiles preferentially living inside lichen thalli and were described as new species for science (*Cladophialophora endolichena* sp.nov. and *Paracaldophialophora lichenicola* sp.nov. in Herpotrichiellaceae, Chaetothyriales, Eurotiomycetes).

Session 4 - Astrobiology and Fungal Dispersal

How can fungi in extreme acid lakes and their salts inform us about possible life on Mars?

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Fungi have been found in some of the most extreme environments on Earth and represents life at habitable lowest water activities. Here, we report common and extremophilic fungi in acid brine lakes in Western Australia with pH as low as 1.4, salinity as high as 32% total dissolved solids, and water activities as low as 0.714. Additionally, halite precipitated from these lakes has yielded fungal cultures that survive desiccation. We propose that extremophilic fungi were thriving in these lakes and common fungi were likely transported into lakes. When halite grew, it trapped fungi both within fluid inclusions and as solid inclusions. Our results show that brines and salts are excellent sinks for indigenous and migrant microorganisms.

Acid brines have been proposed as possible past surface waters and groundwaters on Mars. Some rocks on Mars share similar mineral suites, sedimentary characteristics, and alteration features with terrestrial acid saline lakes in Western Australia and Chile. Besides fungi, these terrestrial environments and their halite and gypsum salts contain Archaea, bacteria, algae, and organic compounds such as beta-carotene. We propose that Mars return samples, in particular any chlorides and sulfates, should be examined for fungi. First, in situ non-destructive methods such as transmitted light and UV-vis microscopy and chemical analyses such as laser Raman spectroscopy, should be conducted to search for fungal suspects, characterize them, and document their context within salt crystals. Then, dissolution of the salt minerals, followed by isolation and culturing should be done to identify genus and species.

Because fungi are known to exist at extreme conditions on Earth, it should be considered a potential past life form on Mars. However, the presence of fungi in a salt mineral is not diagnostic of habitability of the environment in which the salt precipitated. Instead, both indigenous and migrant organisms must be considered.

Fungal conidia prepares for the future according to environmental conditions present before dormancy

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Filamentous fungi produce large quantities of clonal but phenotypically heterogeneous conidia that can stay dormant for a long time until favorable conditions are encountered. How fungal conidia achieve phenotypic heterogeneity and prepare for dormancy is not known. Studies have shown that conidia contain abundant stable messenger RNAs (mRNAs); however, their origin and purpose remain unclear. Our work showed that the so-called dormant conidia of three filamentous fungal species (*Aspergillus nidulans*, *Aspergillus fumigatus* and *Talaromyces marneffeii*) have robust transcription activities to synthesize their own mRNAs. Conidia remain transcriptionally active and responsive to the changing environment until they leave the developmental structure or become dehydrated. These environment-specific transcriptional responses can influence conidial content, expedite gene expression during germination, and affect subsequent fitness and capabilities of the fungal cells including drug and stress resistance, mycotoxin and secondary metabolite production, and virulence. Our findings uncover a mechanism for how genetically identical conidia achieve phenotypic variation and suggest that filamentous fungal conidia prepare for the future by synthesizing and storing transcripts according to environmental conditions present before dormancy.

Mycogenic metal nanoparticles for in situ resource utilization in microgravity environments

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There has been a considerable increase in the number of existing and upcoming space missions. Despite the increased drive towards research associated with such missions, the study of fungi and mycogenic processes of relevance to space exploration remains under-studied.

Fungi have a profound impact on all life on Earth and the same is true for any terrestrial life found outside our planet, e.g., during space exploration. But this fungal impact might be different and altered under extra-terrestrial conditions. Fungal species were found to thrive in space stations and spacecrafts, with some constituting potential health hazards or contribute to infrastructure degradation. Other species can be highly useful for in situ production processes, particularly for long space missions where payload needs to be reduced. Mycogenic processes provide several advantages over other microbial processes: easier manipulation, processing (they do not require cell rupture), and scale-up, as well as reduced costs due to use of cheaper substrates.

Our current research focuses on several fungal production processes to infer on fungal products potential to be used in space exploration for technologies development, new materials, in situ resource utilization, in situ production processes. In this study, we used several different fungal strains, isolated from Mars analogue sites (hypersaline locations in Cabo Verde) and environments with salinity gradients (UK). These were grown in the presence of artificial Mars and Lunar regoliths, and exposed to altered gravity (microgravity – 10^{-3} G and hypergravity – 2G) using a gravity simulator, Gravity Controller Gravitite #GC-JP-RCE01 (As One International, Japan).

The tested strains were analyzed for differences in morphological development and screened for the production of several products (metal nanoparticles and enzymes). Here we show that filamentous fungi open the way towards potential alternative products and materials that will facilitate future space endeavors.

Session 5 - Interactions

Genomes of 14 black fungal lineages isolated from rock-dwelling lichens – a resource for understanding fungal lifestyles and environmental adaptations

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Bare rocks are one of the most inhospitable environments on Earth, due to often constant exposure to UV radiation, heat, drought, rapidly changing environmental conditions, and low nutrient supply. Yet, different organisms, most notably lichens and black fungi, colonize terrestrial rocks everywhere on this planet. The mechanisms enabling black fungi to survive such extreme environmental conditions are still poorly understood, as well as the specific relationships between lichen-associated black fungi and the primary partners of the lichen symbiosis. Here, we use a genomics approach to provide insights into the physiological potential of black fungi. We sequenced genomes of 14 lineages of black fungi isolated from rock-dwelling lichens of the genus *Umbilicaria*. We selected samples from our culture collection, which were phylogenetically diverse, according to their ITS sequences. Long-read sequencing was done on the PacBio Sequel II platform. For de novo assemblies we used HiFi reads based on circular consensus sequencing. The highly contiguous assemblies consisted of 5–42 contigs, had a mean coverage of 79–502x, an N50 of 1.0–7.3 Mb, and BUSCO completeness >95 %. Genome sizes ranged from 26–46 Mb. Phylogenetically, nine of the lineages belong to the Eurotiomycetes (Chaetothyriales) and five to the Dothideomycetes (i.a., Capnodiales). Interestingly, most Dothideomycete lineages are not closely related to any sequenced or known fungi in GenBank. These genomes will be a valuable resource to explore the genome structure and content of black fungi and infer putative interactions between black fungi and the lichen consortium.

For better or for worse: co-habitation of the fungus *Penicillium citrinum* and the yeast *Meyerozyma* sp. in deep geothermal fluids

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Deep geothermal fluids used for heat or electricity production present a combination of conditions that are extreme for life, such as high temperature, high pressure, high salinity, and the presence of heavy metals. The microbial diversity in these fluids is still highly unknown, partially due to the limited access to such environments. However, microorganisms surviving in geothermal fluids, either as spores or as active cells, can have drastic impacts on the power plant systems. Indeed, a sudden change in conditions, such as a temperature drop, may induce microbial growth, which in turn could lead to filters' blockage for instance. In the frame of the REFLECT project, deep fluids from the Heemskerk power plant (NL) were sampled and a mesophilic *Penicillium citrinum* was isolated. The stress tolerance to various abiotic factors of *P. citrinum* spores was tested, which triggered a yeast growth. This growth form appeared first, soon followed by the typical filamentous form of a *Penicillium* fungus. While dimorphy has been previously described in this genus, ITS sequencing of the yeast form revealed that it was affiliated to the genus *Meyerozyma*. Despite several culture purification steps in order to obtain the *Meyerozyma* phase in pure culture, a mycelial form of *P. citrinum* grew out of the apparently pure cultures after prolonged cultivation. This may suggest a co-habitation of *P. citrinum* and *Meyerozyma* sp., with a change of dominance in the medium under abiotic stressful conditions. Interestingly, the analysis of the whole fungal community present in the geothermal fluids from Heemskerk through ITS Illumina sequencing confirmed the presence of both genera (*Penicillium* and *Meyerozyma*) in the power plant system. This co-habitation may be a mechanism to cope with changing environmental parameters (temperature, pressure), allowing these organisms to survive in the extreme conditions of geothermal power plants and their reservoirs.

Session 6 - Radiation, Acidity and Other Extreme Environments

The Role of Mn Antioxidants in Extremotolerant Fungi

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Environmental fungi are a group of highly radiation-resistant eukaryotes. We compared the radiation survivability of 95 wild-type yeast and dimorphic fungal isolates, maintained at the Microbial Culture Collection Ex (Mycosmo), representing diverse Ascomycota and Basidiomycota. The results suggest that resistance to acute and chronic forms of ionizing radiation is a complex phenotype that cannot be predicted by comparative genomics. Instead, evidence is mounting that small high-symmetry antioxidant complexes of manganous ions with metabolites (H-Mn²⁺) are responsible for cellular radiation resistance, and that H-Mn²⁺ protects the proteome, not the genome, from radiation-induced reactive oxygen species (ROS). We show that the amount of H-Mn²⁺ in nonirradiated fungi is readily gauged by absorption-display electron paramagnetic resonance (EPR) spectroscopy and highly diagnostic of their DNA repair efficiency and survival after gamma-radiation exposure. This spectroscopic measure of cellular H-Mn²⁺ content is the strongest known biological indicator of cellular radiation resistance between and within organisms across the three domains of the tree of life. The survival strategy of fungi, like radioresistant *Deinococcus* bacteria, involves the coupling of metabolic and DNA repair functions, resulting in an extraordinarily efficient homologous repair of DNA double-strand breaks (DSBs) caused by radiation or desiccation. The keys to their survival thus lie in the hyperaccumulation of H-Mn²⁺ antioxidants that catalytically remove superoxide, and so protect their DNA repair proteins under extreme oxidative stress, and polyploidy that facilitates DSB repair. This coupling of metabolic and DNA repair functions has made fungi a useful tool in environmental biotechnology, radiobiology, aging, and even planetary protection (<https://www.liebertpub.com/doi/10.1089/ast.2022.0065>).

On the dispersal of fungi between extreme environments

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Many questions about the dispersal of fungi remain unanswered. It is unclear to what extent discoveries in the biogeography of plants and animals can be applied to microscopic fungi and how dispersal is related to the many different reproductive strategies observed in different fungal species. The dispersal of extremotolerant and extremophilic fungi is of particular interest. Some extreme habitats are extremely fragmented, often forming small patches separated by vast distances and temperate environments, such as hypersaline lakes or geothermal springs. Others form large contiguous regions, for example deserts and polar ice sheets. Can fungi migrate between fragmented habitats? Is their distribution in contiguous habitats uniform? Does their ability to thrive in temperate conditions affect their dispersal ability? While direct observation of microbial migration is difficult, population genomics has been able to provide some insight into the dispersal ability of different species from extreme environments. For example, the polyextremotolerant black yeast *Aureobasidium pullulans* forms a well-admixed, intensely recombining global population, while its closely related species *Aureobasidium subglaciale* is strictly clonal. Two other clonal species are *Aureobasidium melanogenum* and the extremely halotolerant black yeast *Hortaea werneckii*. However, these two species occasionally form stable diploid hybrids that can apparently spread over large geographical distances. The ubiquitous basidiomycete *Wallemia mellicola* shows a low level of recombination, but surprisingly so does the halophilic *Wallemia ichthyophaga*, a species that is exceedingly rare and cannot grow under temperate conditions. The diversity of dispersal abilities of extremophilic and extremotolerant fungi seems to reflect the diversity of their reproductive strategies. The implications of these findings for extreme mycology are only beginning to be understood.

Antimicrobial warfare: bacterial-mediated fungal killing through the Type VI secretion system

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Extreme environments arise in polymicrobial communities where fungi and bacteria co-exist, due to numerous antagonistic interactions. Many Gram-negative bacteria compete against their microbial neighbours using the Type VI secretion system (T6SS), which is a membrane-bound nanomachine that fires toxic effector proteins directly into target cells. Whilst a primary role attributed to the T6SS is to deliver antibacterial effector proteins into rival bacterial cells, recently the exciting discovery was made that this 'anti-bacterial' T6SS is also a potent anti-fungal weapon able to kill model and pathogenic fungi by delivering dedicated anti-fungal effectors. We have been focusing on two antifungal effectors elicited by the *Serratia marcescens* T6SS, Tfe1 and Tfe2. Initial studies revealed that Tfe1 intoxication leads to plasma membrane depolarisation, whereas Tfe2 disrupts nutrient uptake and amino acid metabolism. However, their precise mode of action is unknown. Here I will present recent advances in determining the mechanism underlying the potent antifungal activity of these effectors. I'll also discuss how anti-fungal T6SSs may trigger extreme environments for fungal competitors across diverse microbial communities and how that studying anti-fungal T6SSs and their effectors could provide key leads towards development of new antifungal strategies.

The Fungal production of specialized metabolites and proteins is depending on ecological strategy

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Fungi can roughly be divided into having three ecological lifestyle-groups, ruderal selected fungi, stress selected fungi and competition selected fungi. Ruderal selected fungi compete by being pioneers and by fast growth rate, stress selected fungi have adapted to live at more extreme ecological conditions and competition selected fungi compete and collaborate by producing specialized metabolites (SMs), specialized proteins (SPs) and specialized exopolysaccharides (SEP). Since fungi thriving at extremes have few competitors, they generally produce less diverse and complicated profiles of specialized metabolites and proteins. Xerophilic, osmophilic and halophilic fungi often produce some, but not many SMs, but mostly they are accumulated in their ascomata (in Ascomycetes), probably in order not to be ingested by insects, worms, nematodes, mites, and other small animals. Examples are species in *Aspergillus* section *Aspergillus*, which often accumulate large amounts of anthraquinones, bisanthrones, flavoglaucins and echinulins in their ascomata. The common extremophile *Aspergillus halophilicus* from section *Aspergillus* series *Restricti* also produce few SMs in the ascomata, such as asperphenamate and echinulins. Other species live in such extreme conditions, that no SMs have been found (and no gene clusters for SMs have been found), for example in *Xeromyces bisporus*. Examples of SM production will be given for common species of *Aspergillus* and other extremophiles / extremotolerant fungi and also of SPs such as exoenzymes and hydrophobins that can be produced by fungi with different life strategies.

Black fungi on technosphere surfaces: new niches for roof-inhabiting cousins

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Human-made systems, also called “build environment” or “technosphere”, sustain human comfort as well as our industrial activities. These systems have become particularly widespread since the Industrial Revolution, i.e., since the 17th century. At the same time, these technical systems – buildings, monuments, energy production, transformation and transmission, water purification and supply systems - serve as new habitats for living organisms.

Life is ubiquitously present on our planet since a very long time: the Earth is 4.54 billion years old and microbial communities have played a key role on our planet for 3.7 billion years. Once human-made system appeared, microorganisms became an integral part of all types of technosphere infrastructure as well.

Here we will illustrate biosphere-technosphere interactions using a specific example of the black fungi and their impact on the efficiency of solar (photovoltaic) panels. This expanding renewable infrastructure for electricity generation is growing on all continents - and create a specific, arid habitat for stress-tolerant black fungi.

Black fungi were once discovered in hot and cold natural deserts – and now belong to the persistent colonisers of human-made deserts of solar parks. This new niche is evolving an impressive biodiversity. So far more than 60 isolates of black fungi belonging to Arthoniomycetes, Eurotiomycetes and Dothideomycetes were obtained from solar panels in Europe and Americas. Here we will present the analysis of this emerging anthropogenic biodiversity.

Opportunities for future research in the field include quantification of the microbial load on technosphere surfaces – along with characterisation of the corresponding microbial diversity. The strategy of precise measurement and characterisation will enable us to reliably determine the beneficial and harmful functions that living microorganisms play in the functioning of energy-generating systems – and technosphere in general.

The cultivable mycobiota of two karstic caves in Italy: undiscovered taxa and potential biotech applications

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Caves are confined subterranean environments that, being characterized by constant low temperature, oligotrophy and darkness can be considered extreme.

The use of caves for touristic purposes affects the ecological balance of these environments, mining both biotic and abiotic components. Understanding the fungal biodiversity in hypogean ecosystems is important, considering that autochthonous fungi may have the potential to biomineralize metals and may be used as promising agents for bioremediation of polluted sites.

This work aims at describing and comparing the cultivable mycobiota of a touristic cave (Grotta di Bossea) in Italy with the mycobiota of a closely related non touristic one (Grotta di Costacalda), in order to determine the anthropic impact on the subterranean fungal diversity.

Sediment samples of the two karstic caves were collected at progressive distances from the entrance and from the touristic path. Fungi were isolated by applying the soil dilution plate method. Following incubation at different temperatures, the isolates were identified by a polyphasic approach.

Overall, 250 fungal isolates ascribable to more than 50 taxa were found. Most of them belonged to the phylum Ascomycota. The sediments of the touristic cave displayed a richer and more diversified community (224 strains - 78 taxa) in comparison with the sediments collected in the non touristic cave (26 strains - 19 taxa). This difference is probably due to visitors carrying propagules or organic material. Notably, strains of *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *C. pseudocladosporioides* and *Pseudogymnoascus pannorum* were found in both caves, indicating them as possible stable resident components of the subterranean mycobiota. Psychrophilic fungi were abundant in the deepest parts of the caves, while, mesophilic and thermotolerant, were more present in the showcave. Finally, three strains, are still under investigation since they most probably represent new fungal lineages.

Evolution, composition, and biomass degrading capacity of the digestive secretome of 14 halophilic fungi

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A new approach to improved understanding of the biology of halophilic fungi is developed. In focus is comparative analysis of the halophilic substrate-mobilizing digestive-secretome. For this a combination of three methods was used to analyze 14 genome-sequenced species of halophilic fungi. The methods used were: **CUPP**, Conserved Unique Peptide Patterns, providing for peptide-based annotation of enzymes to both protein family and EC function; **EPR**, enzyme profile relatedness; based on CUPP, and including annotation to integrated “Function; Family” observations; and **HotSpot** analysis, based on CUPP and EPR. Using this integrated CUPP, EPR and HotSpot approach, made it possible to identify the halophilic species with the highest digestive enzyme capacity; or the ones with the highest function-specificity diversity. This is done by including total biomass degradation, or by analyzing separately the specific fungal digestive profiles, targeting respectively, cellulose, hemicellulose, pectin, or lignin. Finally, we analyzed all 14 species for their enzyme blend composition, elucidating specifically which types of enzymes are co-occurring in their (wild-type) secretome blend. We call this new tool **Enzyme Blend** analysis. With this method we can e.g., answer the following questions: Is there any specific characteristics in enzyme secretome composition of the species with the highest digestive capacity? Or which enzyme functions are found in the minimal set of biomass-degrading enzymes, present in the species with the lowest digestive capacity? The combined CUPP, EPR, HotSpot and Enzyme Blend analytical approach is shown to provide new insight into the biology of halophilic (and halotolerant) fungi; and, also shown to be efficient in guiding applied, function-targeted, fast track enzyme discovery; opening for identifying the most promising enzymes and most efficient enzyme blends, suitable for high salt industrial processing of biomass.

The extreme of the non-extremophiles: *Aspergillus fumigatus* drug tolerance and persistence

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The phenomena of antibacterial tolerance and persistence, where pathogenic microbes can survive for extended periods in the presence of cidal drug concentrations, have received significant attention in the last decade. Cell responses against antifungals other than resistance have rarely been studied in filamentous fungi, while terms as tolerance and persistence are well-described for bacteria. Several mechanisms of action have been elucidated, and their relevance for treatment failure in bacterial infections demonstrated. In contrast, our knowledge of antifungal tolerance and persistence is still very limited. *Aspergillus fumigatus* is a filamentous fungal pathogen that causes a disease named aspergillosis, for which azoles (AZO), fungicidal drugs, are used as a first-line therapy and caspofungin (CAS), a fungistatic drug, is used as a second-line therapy. In this work, we studied *A. fumigatus* voriconazole persistence and caspofungin paradoxical effect (CPE), a phenomenon where some *A. fumigatus* clinical isolates can survive and grow in CAS concentrations above the minimum effective concentration (MEC). We demonstrate that some isolates of *A. fumigatus* display persistence to voriconazole and a subpopulation of the persister isolates can survive for extended periods in the presence of supra-MIC concentrations of voriconazole. Because all spores derived from an individual strain were phenotypically indistinct with respect to CPE, it is likely that CPE is a genetically encoded adaptive trait that should be considered an antifungal-tolerant phenotype. We propose that the mechanisms which governing these phenomena are multifactorial. We report the identification of several genetic determinants involved in *A. fumigatus* voriconazole persistence and CPE, including transcription factors, the phosphatase calcineurin and mitogen-activated protein kinases (MAPK). We propose that drug tolerance and persistence are important factors to consider and further investigate in *A. fumigatus* and they can help the fungus in the way to the emergence of drug resistance.

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Comparative Genomics of Thermophilic Fungi

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A genome collection of over 30 thermophilic and thermotolerant fungi has been sequenced and assembled in the U.S. Department of Energy Joint Genome Institute's MycoCosm portal (<https://mycocosm.jgi.doe.gov>) to understand molecular basis of thermophily using comparative genomics. These fungal traits are scattered across the fungal tree of life with their highest concentration within species of Chaetomicaceae, Thermoascoceae, and Trichomaceae as well as some Mycoromycota. Thermophilic genomes encode a range of thermostable enzymes including carbohydrate-active enzymes such as xylanases, useful for many industrial applications. At the same time, the overall gene counts, especially in gene families responsible for microbial defense such as secondary metabolism, are reduced in thermophiles in comparison to mesophiles. Comparative genomics analysis also revealed higher GC3 and lower effective number of codons in fungal thermophiles than in thermotolerant and mesophilic fungi. Furthermore, using the Support Vector Machine classifier we were able to identify several Pfam domains capable of discriminating between genomes of thermophiles and mesophiles with accuracy > 0.88. Thus, comparative genomics offers new insights into biology, adaptation, and evolutionary history of thermophilic fungi while providing a parts list for bioengineering applications.

Fungal diversity and its geochemical role in the extreme acidic environment of Río Tinto

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Río Tinto (SW Spain) is an extreme environment with a noticeable high concentration of toxic heavy metals. But what makes Río Tinto a unique extreme acidic environment is the unexpected degree of eukaryotic diversity detected in its basin, being fungi the most diverse among decomposers. The aim of the present study was to isolate and describe the seasonal diversity of the fungal community in the Tinto basin and its correlation with the physico-chemical parameters existing along its 100 km course. Molecular diversity studies were performed using the complete ITS regions. The taxonomic affiliation showed a high degree of diversity, identifying species belonging to Ascomycota, Basidiomycota and Zygomycota, being the former the most common of the fungal isolates. Selected isolates were evaluated for their tolerance to toxic heavy metals. Some isolates showed a high tolerance to Cu^{2+} , As^{5+} , Cd^{2+} , Co^{2+} , Ni^{2+} and Pb^{2+} and an important role in biomineralization processes. The geochemical role of acidophilic fungi in the Tinto basin will be evaluated.

Battle of extremophile *Rhodotorula kratochvilovae* strains for best process metrics in simultaneous detoxification and lipid production from steam explosion hydrolysate

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The carotegonic yeast *Rhodotorula kratochvilovae* is known as a versatile Basidiomycota that can utilize a wide range of substrates, including waste materials, and produce a wide range of high value biomolecules. The challenge in our research is to use this species to detoxify the inhibitory lignocellulose-derived hydrolysate from steam-exploded poplar and simultaneously convert it into triacylglycerides (TAGs). To this end, we screened five *R. kratochvilovae* strains from the Mycosmo Ex Culture Collection, Slovenia, and compared their performance with *Cutaneotrichosporon oleaginosum* ATCC20509. Contrary to the state of the art, we did not use any additional carbohydrate substrate.

R. kratochvilovae strains EXF3471, EXF3697 and EXF9590 were obtained from glacial ice, EXF11626 from a wasp nest and EXF7516 from the air. At 30°C, there was no trend in the fatty acid composition of TAGs, TAG amount, enzyme production or substrate conversion between the group of glacial origin strains and the other strains. Regarding process metrics, *R. kratochvilovae* strains generally outperformed *C. oleaginosum* in terms of growth rate, as well as consumption rate and total consumption of the phenolics, furans and organic acids. The conditions did not allow a high production of TAGs as most of the non-monomeric substrate (measured as dissolved organic carbon) was not degraded, although it was shown that lignin and manganese peroxidase, cellulase and xylanase were produced by most of the strains. The conditions applied also showed that not only nitrogen and phosphate depletion induced lipid accumulation, but that oxygen limitation also had the same effect. This novel aspect can be explained by the fact that the yeasts cope with the low oxidative power by reducing substrates to TAGs instead of metabolizing them during growth.

Although further optimization is needed, *R. kratochvilovae* strains show high biotechnological potential in detoxification and valorization of phenolic acidic waste streams.

Exploiting the black yeast *Aureobasidium pullulans* for poly(malic acid) production

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Aureobasidium pullulans is a highly versatile yeast-like ascomycete. As a ubiquitous saprophyte found in diverse environments, *Aureobasidium* exhibits a high tolerance towards extreme pH values and salt concentrations. Furthermore, *Aureobasidium* produces a broad spectrum of biotechnologically relevant secondary metabolites like the biopolymer poly(malic acid) (PMA). In addition, the fungus can grow on a wide range of carbon sources, including renewable raw materials and waste products, making it an attractive, robust platform organism for future applications in a circular bio-economy. PMA, a linear anionic polyester, and its derivatives have a broad spectrum of prospective applications, e.g., in medicinal products or as bioplastics.

In this study, we aimed to identify the metabolic pathway employed for PMA monomer (malate) synthesis, the enzyme(s) responsible for polymerization, and to increase the efficiency of PMA production by metabolic engineering.

- 1) We first identified three possible pathways for malate synthesis *in silico*. Then, in gene knock-out experiments, we could show that one of these is the dominant pathway of malate synthesis. The respective knock-out strain showed a 61% decreased PMA yield.
- 2) Regarding polymer formation, knocking out a certain gene cluster completely abolished PMA production while biomass formation increased.
- 3) To enhance PMA production efficiency, we again used a gene knock-out strategy to avoid the synthesis of the three most abundantly produced by-products. While for two by-products, PMA production was not significantly affected, the deletion of biosurfactant production increased the PMA yield by a factor of 2.2.

In shake flask experiments, the best performing strain, produced 19.3 g/L PMA with a yield of 0.25 g/g glucose.

In summary, we were able to elucidate the PMA synthesis pathway in *Aureobasidium pullulans*, including monomer synthesis and polymerization, and doubled PMA production efficiency by employing genetic engineering.

Green-light sensors in extremophilic fungi

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Light plays a crucial role in many significant processes in the fungal life cycle, including reproduction and pathogenicity, making photoreceptors essential in many fungal species. Among the various fungal light sensors that react to different wavelengths, fungal rhodopsins are responsible for detecting green light. As type I (microbial) rhodopsins, fungal rhodopsins consist of seven transmembrane helices that surround the covalently bound chromophore all-trans retinal. Upon light activation, retinal undergoes a conformational change that provides protein function. Fungal rhodopsins can function as proton pumps or sensory proteins, but the details of their physiological function and biological role are still limited. We highlight the role of these green light sensors in fungal biology, especially in the green-light-enriched phyllosphere. We report on the biophysical characteristics of different rhodopsins and the increase of proton-pump activity observed in certain fungal rhodopsins upon stimulation with the plant auxin indol acetic acid. The genome of the extremophilic fungus *Aureobasidium pullulans* exhibits three genes coding for putative rhodopsins ApRh1, ApRh2, and ApRh3. After heterologous expression of these genes in mammalian cells and *Xenopus* oocytes, all three proteins exhibited proton pump activity with maximal activity in green light, with ApOps2 exhibiting the most pronounced proton pump activity. We also used site-directed mutagenesis to reshape protein function and implement light-gated proton channels. Additionally, we report on our investigation of rhodopsins from other extremophilic fungi.

Session 7 - Black Fungi

Evolution of opportunism in Chaetothyriales

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Fungi in the order *Chaetothyriales* are renowned for their ability to cause human infections. Nevertheless, they are not regarded as primary pathogens, but rather as opportunists with a natural habitat in the environment. Extremotolerance is a major trend in the order, but quite different from black yeasts in *Capnodiales* which focus on endurance, an important additional parameter is advancing toxin management. In the ancestral ecology of rock colonization, the association with metabolite-producing lichens is significant. Ant-association, dealing with pheromones and repellents, is another mainstay in the order. The phylogenetically derived family, *Herpotrichiellaceae*, shows dual ecology in monoaromatic hydrocarbon assimilation and the ability to cause disease in humans and cold-blooded vertebrates. In this study, data on ecology, phylogeny, and genomics were collected and analyzed in order to support this hypothesis on the evolutionary route of the species of *Chaetothyriales*. Comparing the ribosomal tree with that of enzymes involved in toluene degradation, a significant expansion of cytochromes is observed and the toluene catabolism is found to be complete in some of the *Herpotrichiellaceae*. This might enhance human systemic infection. However, since most species have to be traumatically inoculated in order to cause disease, their invasive potential is categorized as opportunism. Only in chromoblastomycosis, true pathogenicity might be surmised. The criterion would be the possible escape of agents of vertebrate disease from the host, enabling dispersal of adapted genotypes to subsequent generations.

Cerebral phaeohyphomycosis

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Cerebral phaeohyphomycosis (CP) is an extremely rare and frequently fatal fungal infection caused mainly by neurotropic dematiaceous fungi belonging to the fungal family Herpotrichiellaceae (order Chaetothyriales), specifically *Rhinocladiella mackenziei*, *Fonsecaea monophora*, *Exophiala dermatitidis*, *Cladophialophora bantiana* and *Verruconis gallopava*. These fungal pathogens are aggressive, exclusively invading the brain tissue likely via the blood-brain barrier, presumably after inhalation, although the portal of entry has not been established with certainty. Cell wall melanin and thermotolerance, which are shared by numerous black fungi, along with the assimilation of alkylbenzene hydrocarbons, which are structurally similar to neurotransmitters, have been suggested to be specific to the Herpotrichiellaceae family of fungi. Although the environmental niches of these fungal pathogens are largely unknown, the gene composition and metabolic potential of *R. mackenziei* indicate extremotolerance and hydrocarbon assimilation, suggesting a possible environmental habitat of oil-polluted desert soil. In the majority of identified cases, CP has resulted in brain abscesses in patients with no predisposing factors or immunodeficiency, with symptoms that have included headache, seizures, cerebral irritation, fever and neurological deficits. Often, the clinical symptoms are misdiagnosed as cerebral tumours or bacterial brain abscesses. CP has a regional prevalence and a high mortality rate of up to 70% with better outcomes observed in patients who undergo complete surgical debridement, antifungal combination therapy and immunotherapy; however, it is refractory to most antifungal agents. If the infection remains untreated, mortality can be 100% within weeks, months or years. The optimal therapeutic regimen for the treatment of CP is unknown. In most clinics, an antifungal regimen with a combination of liposomal amphotericin B and triazoles (itra-, vori- or posa- conazole) is also used as an initial therapy.

Omics analysis of *Cladophialophora exuberance* focusing its potential on bioremediation of hydrocarbon and heavy metal polluted habitats

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Cladophialophora exuberans is a filamentous fungus related to black yeasts in the order Chaetothyriales. These melanized fungi are known for their 'dual ecology', often occurring in toxic environments, and being frequently involved in human infection. Particularly *Cladophialophora exuberans*, *C. immunda*, *C. psammophila*, and *Exophiala mesophila* have been described with a pronounced ability to degrade aromatic compounds and xenobiotic volatiles, as benzene, toluene, ethylbenzene, and xylene and are candidates for bioremediation applications. The sequencing, of the *C. exuberans* whole genome focused on genes and pathways related to carbon and toxin management, assessing the tolerance and bioremediation of lead and copper, and verified the presence of genes for metal homeostasis. Genomic evaluations were carried out through a comparison with sibling species including clinical and environmental strains. Tolerance of metals was evaluated via a microdilution method establishing minimum inhibitory (MIC) and fungicidal concentrations (MFC), agar diffusion assay and heavy metals bioremediation was evaluated via graphite furnace atomic absorption spectroscopy (GFAAS). The final assembly of *C. exuberans* comprised 661 contigs, with genome size of 38.10 Mb, coverage of 89.9X and a GC content of 50.8%. In the agar tests, the strain grew at 2500 ppm of copper and lead. In GFAAS assays, it was observed an uptake capacity of 89.2% and 95.7% of copper and lead, respectively, after 21 experimental days. In addition, the *C. exuberans* showed inhibition of growth at concentrations of 1250 ppm for copper and at 625 ppm of lead, using the MIC method. This study enabled the annotation of genes involved in heavy metal homeostasis and contributed to a better understanding of the mechanisms used in adaptation to extreme conditions and to protect itself.

Melanin Excretion from the Novel Black Yeast *Exophiala viscosa*

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Exophiala viscosa is a newly characterized fungus isolated from a biological soil crust in the Canadian Rockies. This fungus grows in xeric, nutrient deplete environments implying highly flexible metabolism and the potential to form lichen-like mutualisms with nearby algae and bacteria. However, the exact ecological niche and interactions between this fungus and its surrounding community is not well understood. A combination of whole genome sequencing, melanin regulation, and microbial interaction experiments have been performed to fully characterize this fungus and help decipher their fundamental niche within the biological soil crust consortium. Our results reveal that *E. viscosa* excretes melanin into its environment, which can provide increased abiotic resistances, and potentially a carbon source, to the biological soil crust community. Our study also provides new insights into the regulation of melanin production in polyextremotolerant fungi, and novel data about this fungus' interaction with algae and cyanobacteria.

Generation of *Cryomyces antarcticus* mutants to explore the importance of DHN melanin for survival

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Cryomyces antarcticus, a cryptoendolithic melanized fungus endemic to Antarctica (phylum Ascomycota, class Dothideomycetes *incertae sedis*), has demonstrated high capability to survive extreme environmental conditions like those found in space (e.g., ionizing radiation, vacuum, microgravity), thus fueling fundamental astrobiological questions like “the search for life beyond Earth”. Its extraordinary resilience has been attributed to the presence of thick, highly melanized cell walls, which may contain both DHN and DOPA melanins. To better understand the contribution of DHN melanin to the overall resilience of *C. antarcticus*, we decided to generate melanin-deficient mutants by genetic engineering. For this, the melanin-PKS (polyketide synthase)- encoding ortholog was identified in the *C. antarcticus* CBS 116301 genome and used to design primers for re-sequencing of the *capks1* locus in the strain CCFEE 515. Based on the genetic toolkit developed for the black fungus *Knufia petricola*, we designed a strategy for mutating *capks1*. Protoplasts of *C. antarcticus* CCFEE 515 were generated and co-transformed with a circular AMA-containing plasmid for expression and *in-vivo* assembly of Cas9 and two *capks1*-specific sgRNAs and a PCR-generated donor DNA *i.e.*, a hygromycin resistance cassette flanked by ~75-bp-long sequences homologous to the *capks1* locus. Transformation of *C. antarcticus* is challenging because of its very slow growth and uncertain gene copy number. It took six months from obtaining enough biomass for cell wall lysis to transferring the putatively resistant transformants for genotyping, but we eventually managed to generate three independent non-melanized (whitish) $\Delta capks1$ mutants! We are now awaiting first complemented strains as ultimate genetic transformation control. They are considered to have a wild-type-like pigmentation due to the reintroduction of the wild-type *capks1* into the $\Delta capks1$ mutant.

Contribution of DHN melanin to survival and growth of *Neoarthrinium moseri* in extreme environments

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Epiphyte *Neoarthrinium moseri* is a sordariomycete isolated from tropical forests in Colombia and Brunei.

Whole genome analysis revealed a high potential for secondary metabolite (SM) production and cultivation on different media resulted in the expression of different yet unknown SMs. Additionally, microbiological assessment using different types of stress factors (salinity, pH, temperature) indicated tolerance of the fungus to extreme environments.

Under certain laboratory conditions, *N. moseri* forms black and tear-shaped conidia. Its close relative *Pestalotiopsis fici* also forms black conidia. We were able to identify the Biosynthetic Gene Cluster responsible for the expression of 1,8-dyhydroxynaphthalene (DHN) melanin in *N. moseri* by comparing genomic data of *N. moseri* and *P. fici*. Deletion of the according polyketide synthase resulted in loss of black color and tear-like shape of the conidia. However, in both strains DHN melanin-deficiency is not lethal.

In this work we investigated the contribution of DHN melanin to the tolerance of *N. moseri* to extreme environments. In addition to the already used test conditions for microbiological assessment (salinity, pH, temperature), we tested the wildtype and the DHN-melanin deficient strain for their susceptibility to among others oxidative and heavy metal stress.

***In-vivo* mutagenesis of the rock inhabitant *Knufia petricola* by a customized *Ac/Ds* transposon system**

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Microcolonial black fungi ubiquitously inhabit sun-exposed natural and man-made surfaces of our planet. To promote genetic studies, CRISPR/Cas9-based genome editing was implemented in the rock-inhabiting fungus *Knufia petricola* (Eurotiomycetes/ Chaetothyriales). Now efficient targeted mutagenesis of *K. petricola* - as a representative of the polyphyletic group of black fungi - enables the elucidation of extremotolerance, oligotrophism, unusual types of cell division, mineral weathering and symbiotic interactions. Even more progress on assigning functions to yet unknown genes can be achieved by a forward genetics approach. We chose the two-component *Activator/Dissociation (Ac/Ds)* transposon system from maize for generating *K. petricola* insertional mutants by *in-vivo* mutagenesis. For the optimal use of this genetic tool, an inducible promoter i.e, from the metabolism-independent Tet-on system, was combined with the *ActPase*-coding sequence enabling the regulatable transposition of the resistance cassette-containing *Ds* transposon. In total, six auxotrophic *Ac/Ds* starter strains containing the *Ds* transposon at different position of *ade2*, *ura3* or *ppt1* were generated. The cultivation of these strains with doxycycline for induction of TET::*Ac* and subsequent selection of cells on ADE/URA/LYS-lacking media resulted in prototrophic colonies (revertants) for most *Ac/Ds* strains. Amplicon sequencing of excision sites revealed characteristic footprint patterns, proving that the transposon jumped. For identifying unknown *Ds* re-insertions sites, the thermal asymmetric interlaced (TAIL)-PCR was successfully implemented. First identified *Ds* re-insertion sites suggest that the distribution pattern may depend on the excision site. Currently, transposition frequencies and genome-wide distribution of re-insertion sites are studied in different *Ac/Ds* starter strains to identify the best candidate for generating saturated mutant libraries. This transposon mutagenesis strategy is also interesting for studying other black fungi, because once the *Ac* and *Ds* components are integrated in the genome, the fungus 'only' needs to be cultivated for generating insertional mutants.

Session 8 - Omics and Molecular Tools for Extremophilic Fungi

Genomic and Phenotypic variation in *Rhodotorula* species sampled from Extreme Environments.

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Rhodotorula are basidiomycete yeasts, which are characterized by production of carotenoids and are found in a broad range of temperature and harsh environments. They are found in spoiled food, in freshwater lakes, ocean and brackish waters, the human built environment, and from soils and rocks in arid lands including cold arctic/antarctic and hot deserts. Some lineages have been characterized from rock surface or endolithic samples. The species *Rhodotorula mucilaginosa* is capable of causing disease in mammals through skin and blood-borne infections. We characterized phenotypic traits of 288 strains across extreme conditions, including temperatures from 4 to 37 C, various carbon sources such as glycerol and xylose, and pH and salinity conditions. The results establish a vast phenotyping dataset consisting of growth curves, growth rates, carotenoid production, and morphological traits. Using short-read sequencing, we have assembled draft genomes from more than 200 isolates from a diversity of temperature and extreme environments to examine the pangenome of the *Rhodotorula* genus, and focused population studies within *R. mucilaginosa*. These comparative analyses enable us to test hypotheses about gene-level adaptation to cold, hot, and saline environments. In addition, we have generated long-read genomes from 9 species of *Rhodotorula*, focusing on type strains, to establish a high quality reference collection of genomes for the genus.

Genetic engineering of black fungi: lessons learned from *Knufia petricola*

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The exponential rise in the number of fungal genomes sequenced by next-generation sequencing techniques makes it necessary to increase efforts to correctly annotate and assign gene functions. There are two possibilities to explore a genome and its gene functions. The hypothesis-based method proves the function of already existing gene/allele candidates by targeted mutagenesis - so called reverse genetics. The basis of forward genetics approaches is the random mutagenesis of the genome, followed by screening of obtained mutants for the phenotype of interest, and identification of the mutated genes in the respective mutants. This strategy is hypothesis-generating, means it is necessary to verify the relationship between the detected mutations and the observed phenotype by targeted mutagenesis of the identified gene. We developed a toolbox for editing the genome of the rock inhabitant *Knufia petricola* [Eurotiomycetes, Chaetothyriales] that allows the study of the phenotypic characteristics of black fungi such as the regulation of pigment synthesis, general stress responses, oligotrophy, and the unusual modes of cell division by advanced reverse and forward genetics approaches. The toolbox includes the annotated genome sequence of strain A95, efficient strategies for CRISPR/Cas9-based genome editing and live-cell imaging using genetically encoded fluorescent proteins, as well as protocols for -omics approaches and for simulation of mineral weathering. A forward genetics approach using transposon mutagenesis is currently developed for identifying essential genes. The established protocols and knowledge gained from *K. petricola* form a starting point for making other fungi from extreme environments accessible to genetic manipulation.

The genome of the polyextremophilic yeast, *Naganishia friedmannii*, reveals adaptations involved in stress response pathways, carbohydrate metabolism expansion, and limited DNA repair repertoire

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Naganishia friedmannii is a basidiomycete yeast commonly found in some of the most extreme environments of the Earth's cryosphere. We isolated *N. friedmannii* from soils > 6000 m above sea level on Volcán Lullullaico, Chile. This yeast has a genome size of 22.2 Mb and its annotation identified 6251 protein coding sequences. Genome analysis revealed the presence of genes that encode for proteins known to be associated to the main stressors to which *N. friedmannii* is exposed in extreme environments, such as genes to cope with thermal, osmotic and radiation stress. Comparative analysis with 8 additional *Naganishia* genomes revealed unique features that help explain the polyextremophilic lifestyle of *N. friedmannii*. Out of the predicted protein coding sequences for *N. friedmannii*, approximately ~12% of the genes were unique to the *N. friedmannii* genome, the majority of those annotated falling in the carbohydrate metabolism and transport category. The comparison of amino acid frequency with closest members outside of the *Naganishia* clade revealed a significant enrichment of glutamine and alanine. A significant preference in flexible and small amino acids may be a key feature in *N. friedmannii* to facilitate catalytic activity at low temperatures and decrease the energetic cost of conformational change required in fast changing temperature environments. Surprisingly, many genes involved in cell-cycle checkpoints and DNA repair processes are missing from the genome of *N. friedmannii*, as well as from several other *Naganishia* species. This extensive loss may be adaptive in extreme environments prone to stressful abiotic changes, where a high mutation rate may provide the opportunity for generation of advantageous traits and reduced cell cycle control may allow for faster reproduction. To determine the evolutionary impact of DNA repair gene loss it will be necessary to experimentally verify whether *N. friedmannii* and related *Naganishia* spp. are subjected to increased mutation rates.

Session 9 – Biotechnology and Bioremediation

Isocyanides as Tools of Survival: Unveiling Fungal Strategies for Copper Starvation

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All organisms have a requirement for the transition metal copper at just the right amounts. Copper extremes in either direction - starvation or toxicity – result at best in ill health or, more typically, death. It is well known that fungi address these extremes through copper homeostasis pathways that involve import or export of copper ions and in some cases use of copper metallothioneins. Here we present unique contributions of fungal isocyanide natural products in mediating fungal success during copper starvation. Rather than involving import or export of copper, these isocyanides provide competitive fitness for the producing fungi against other microbes in low copper environments. The isocyanide xanthocillin chelates copper thus preventing other microbes from accessing copper ions and products of the isocyanide synthase CrmA – either only produced or increased in production during copper starvation – exhibit antimicrobial properties giving the fungus a survival advantage over other microorganisms. We present a bioinformatic view of the newly discovered isocyanide biosynthetic gene clusters in fungi and discuss a prospective of their role(s) in metal extreme environments.

Applications of isolated fungi for bioremediation purposes

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In the Anthropocene, fungi represents a group of microorganisms able to perform singularly useful reactions in many fields of interest for humans, principally in biotechnology and bioremediation. Although many biochemical fungal features are known, still many taxa and groups remain unexplored, mainly those who has special adaptation to particular environments, including extremely anthropogenic environments (anthropophilic microorganisms). Fungi isolated from different environments have shown their ability to remove chemicals of very different nature, including aromatic compounds and/or hydrocarbonated chains. In this study, we show different examples of the use of fungi isolated from arid environments, PAHs-polluted areas and sewage sludge thermophilic composting piles. Here we describe; the capacity of oxy-functionalization of different aromatic compounds by some representative molecules such as polycyclic aromatic hydrocarbons (PAHs), pharmaceutical active compounds (PhACs: non-steroidal aromatic compounds); passivation of heavy metals (Cu, Zn, Pb and Ag); adaptation to poly-contamination PAHs-HM; as well as functional changes in plastics -polyethylene terephthalate, high-density polyethylene, polyvinyl chloride, low-density polyethylene, polypropylene- and conductive paper sensors using different technologies (UHPLC-QToF, ICP, SEM, FTIR, AFM). Most of the hydroxylation reactions lead to less toxic compounds (marked by microtoxicity reduction) through their complex enzymatic machinery, in the majority of the cases mediated by the intracellular system (Phase I and II of the detoxification system).

These results emphasize the prominent potential of fungi associated with the microbiota of anthropogenic environments as catalysts for several important reactions involving organic and inorganic molecules.

Non-conventional yeast: Investigating their potential for treatment of fish farm effluents and agro-industrial wastewaters

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The utilization of microorganisms to produce biochemicals and biofuels from renewable resources offers many advantages in terms of environmental sustainability. However, the major priority is the identification of strains able to grow on natural sources and/or waste streams and convert such types of raw feedstock into bioproducts. In this context, non-conventional yeasts are attracting increased attention in biotechnological applications thanks to their natural characteristics, such as thermo- and osmo-tolerance, salt resistance, and their ability to metabolize complex carbon sources. Still, research exploring their applications is still scarce. The purpose of this study was to identify non-conventional yeast strains capable of thriving in oligotrophic environments with high salinity and/or pH values at different temperatures, aiming to utilize them in wastewater treatment. Thus, we cultivated 1065 strains preserved in the Microbial Culture Collection EX-Mycosmo specialized on halophilic and halotolerant yeasts, in liquid medium derived as residual after microalgae cultivation on fish farm effluents (FFE), at temperatures of 15, 25, and 37 °C. Interestingly, 10.0, 8.1, and 25.7%, respectively, exhibited satisfactory growth. The strains able to grow at 15 and 25 °C were further cultivated on FFEs enriched with pomegranate residues (PRs), which served as a carbon source containing 22.3 g/L of reducing sugars. Among these, 21.9% grew at 15 °C, while 25.8% thrived at 25 °C. This finding suggests the potential for simultaneous treatment of two types of wastewaters in Northern and Mediterranean European countries. Additionally, the strains that grew at 37 °C on FFEs, which are typically categorized as opportunistic pathogens, were further cultivated in blends of FFEs and olive mill wastewaters (OMWs), an agro-industrial residue rich in phenolic compounds (i.e., 5.3 g/L). Remarkably, 33.3% of them were able to grow under these conditions. In conclusion, non-conventional yeasts offer new applications on the valorization of wastewaters.

KEY NOTE LECTURE

Water, water everywhere, nor any drop to drink

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Water is the most important life-limiting factor on planet Earth. Most microorganisms need large quantities of water to survive, while some extremotolerant microorganisms have evolved adaptations that enable them to live at extremely limited water conditions. Water can be limited even when it appears plentiful at first sight. It can be chemically bound, as in hypersaline environments, it can be frozen, as glacial ice, at high temperatures it can be aerosolized or simply scarce, as in arid environments. All these at first sight very different and inhospitable environments, have one characteristic in common - low water activity and frequently high concentrations of osmolytes. Nevertheless, they are globally inhabited by diverse extremotolerant and extremophilic fungi. Some fungi are specialized in only one of these environments, while most are generalists and highly adaptable. Both groups efficiently balance cellular osmotic pressure and ion concentration, stabilize cell membranes, remodel cell walls, and neutralize intracellular oxidative stress. Some species use new and unusual reproductive strategies. While biodiversity in most of these environments is relatively known, the ecological functions of this fungal diversity are largely unexplored. More research is also needed in the face of climate change. On one hand they can contribute to global adaptations of fungi to life at higher temperatures and on the other hand drive additional species into environments where they were so far scarce, such as households. Consequences can range from an increase in the number of opportunistic pathogenic fungi both in nature and in domestic environments, to massive losses in biodiversity in rapidly changing cryosphere. Extremotolerant fungi may be directly affected by these processes, but they can also help shape them.

POSTERS

Session 1 - Rock-Inhabiting Fungi

STRES project delivers eleven new draft genomes from never sequenced genera of melanized Eurotiomycetes and Dothideomycetes isolated from lichens

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The polyphyletic group of stress-resistant black fungi within Ascomycota is ubiquitous in natural and anthropogenic habitats. Among other adaptations, their melanized cell wall makes them one of the most versatile poly-extreme-tolerant organisms. The STRES project (Shedding light on the dark lineages of the fungal tree of life; <https://stresblackfungi.org>) focused on unsampled genera that display different ecologies and lifestyles, resulting in the annotation of approximately 60 new draft genomes of black fungi. Specifically, in rocky environments, lichens are often the most abundant primary producers, and they have recently been also emphasized as a source of hidden fungal diversity. Therefore, we focused on this undersampled niche, isolating melanized fungi from lichen thalli. The isolated strains were initially characterized using rDNA ITS and LSU barcoding. Subsequently, high-quality genomic DNA from eleven of these strains, belonging to five genera, was sent to the DOE Joint Genome Institute and sequenced using Illumina technology. Eleven draft genome assemblies were generated and assessed for completeness and contiguity. The resulting assemblies were compared with each other and with genomes from related fungi exhibiting different lifestyles, employing a comparative genomic approach to gain insights into genomic features of lichen associated extreme-tolerant black fungi. The availability of new genomes from previously unsequenced taxa can contribute both to resolve the fungal tree of life and to highlight biotechnologically promising strains.

Characterization of the community of Rock Inhabiting Fungi associated to white marble of Florence Cathedral

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Rock-inhabiting fungi (RIF) represent one of the most damaging groups of microorganisms causing deterioration of outdoor exposed stone monuments. In addition to chromatic alteration, they can cause micropitting and stone pulverization due to their ability to grow in the micro-porosity and cracks of the rocks. Their removal and treatment are challenging for restorers because of their (poly)extremotolerant features that lead to high resilience.

In the present work, we studied the community of RIF inhabiting the external white marble of the Cathedral of Santa Maria del Fiore in Florence (Italy). In a previous work they were demonstrated to be responsible for the darkening of this marble. Twenty-four morphologically different strains were isolated from marble of two differently exposed sites of the Cathedral and characterized. A phylogenetic analysis based on the sequences of ITS and LSU rDNA regions was performed and showed a wide diversity of the RIF in the limited sampled areas. The isolates belonged to five known genera: *Knufia* and *Lithohypha* (class Eurotiomycetes), *Coniosporium*, *Paradevriesia* and *Vermiconia* (class Dothideomycetes). Two additional strains of Dothideomycetes would be novel fungi since an identification at a deeper taxonomic level than class was not possible by similarity with known sequences.

Physiological tests were conducted to investigate the interactions of RIF with the stone substrate. In particular, eight strains belonging to different genera were tested for acid production to highlight the potential chemical damage they could cause by growing on carbonatic stones. Moreover, their sensitivity to conventional and innovative biocides was tested. We used the commercial biocide Biotin T and the essential oils of thyme and oregano with the aim of finding natural and safe compounds, with a low environmental impact, active against these fungi.

Session 2 - Fungi in Low Water Activity Environments

Draft genome annotation of the oosporein-producing *Victoriomyces antarcticus* strains MUT3686, MUT5898 and MUT5937 (Sordariomycetes, Cephalothecaceae) isolated from Antarctic soils and African desert oases

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Victoriomyces antarcticus strains MUT3686 and FBL577 (Cephalothecaceae) were isolated from Antarctic soils. More recently, two other strains of the same species (MUT5937 and MUT5898) were isolated from soils collected from oases in the Tunisian desert. Noteworthy, all *Victoriomyces* strains found both in Antarctic and African extreme habitats showed a production of a diffusible red pigment. In this study, the red pigment extracted from culture filtrate of MUT3686, MUT5898 and MUT5937 was identified by HPLC as the secondary metabolite oosporein, a symmetrical 1-4 dibenzoquinone pigment, which could be relevant in challenging environments. Furthermore, MUT5937 was shown to produce significantly less oosporein than the other two strains. Here, we report the genome of MUT3686, MUT5898 and MUT5937 sequenced with short- and long-read sequencing technologies. Moreover, we sequenced *Cryptendoxyla hypophloia* MUT1750 and

Acremonium thermophilum MUT6595 (= CBS 734.71) belonging to Cephalothecaceae to provide a basis for comparative study of this intriguing family. Mining the genome of the Cephalothecaceae lineages examined here revealed different ecological traits, as well as the biosynthetic gene cluster (BGC) of MUT3686, MUT5898 and MUT5937 potentially involved in the oosporein synthesis. We hypothesize that the oosporein biosynthesis in *Victoriomyces* strains begins with a PKS to synthesize orsellinic acid, with oosporein being transported across the cell membrane by a protein encoded in the BGC. Regulatory proteins outside the BGC may be involved in the regulatory mechanism of oosporein synthesis. However, by analyzing sequence of the GAL4 transcription factor located in the oosporein BGC we have identified a basis for understanding the levels of oosporein produced by *Victoriomyces* strains. Finally, the oosporein BGC detected in *Victoriomyces* strains had sequence homology/synteny with the oosporein BGC/PKS cluster present in Hypocreales and Xylariales genomes, allowing to hypothesize horizontal gene transfer events.

Dormancy of extremotolerant *Parengyodontium torokii* in salt crystals

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Over the last two decades, it has become increasingly clear that extremophilic fungi have pushed the borders of the sustainability of eukaryotic life. Here, we report on the isolation of fungi from two acid (pH down to 1.4%), saline (up to 32%) total dissolved solids (TDS) and magnesium-rich lakes in Western Australia: Lake Magic and Lake Gneiss. These remote, ephemeral lakes are characterized by flooding-evapoconcentration-desiccation cycles driven by the local hot and dry climate. Their complex precipitation dissolution chemistry depends on long periods of water-rock interactions, shorter flooding cycles followed by desiccation, when lake beds become salt pans covered with salt crusts. These relatively short-lived cycles may last from hours to months. Fungi were isolated from samples of water, microbial slime, and halite crystals, that were stored in refrigerator from 4-10 years. The dominant fungus, represented by nine strains, was a newly described species *Parengyodontium torokii* N.K. Singh and K. Venkateswaran (Parker et al. 2022). *P. torokii* isolates were tested for survival after desiccation and for salt tolerance on media containing osmotic, stabilizing salts (NaCl, KCl, and MgSO₄) as well as to chaotropic, destabilizing salts (NaBr, MgCl₂, and CaCl₂). Isolates were also exposed to saturated solutions of these salts in liquid media and exposed to evaporation and crystal formation. Our preliminary results suggest that crystals of halite and other crystals may preserve *P. album* in a dormant form, either adhered to salt crystals or as inclusions within, since after dissolution of crystals, the fungal strains resumed metabolic activity.

Comparative study of the fungal biota of a hypersaline and a freshwater lagoon in Spain

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In the last years, several studies about the microbial composition of extreme environments have been carried out worldwide; however, only a few of these studies have been focused on the analysis of fungal ecosystems in saline lagoons in Europe. "Las Saladas de Chiprana" is a complex of lagoons located in Zaragoza province (Spain), and has the only hypersaline lagoon of permanent and deep waters in Western Europe ("Salada Grande"). For this reason, the aim of the present study was to analyze the fungal diversity of the sediments of "Las Saladas de Chiprana" and to compare the results with a nearby non-salty lagoon, "La Estanca", by a metabarcoding analysis using the internal transcribed spacer 2 (ITS) region. As a results, a total of 764,536 sequences were identified, which were classified into 2,075 Amplicon Sequence Variants (ASVs). However, 1,464 ASVs were only identified at the kingdom level (70% of the total). The number of ASVs detected in the lagoon "La Estanca" (636 ASVs) was significantly higher than those obtained in "Salada Grande" (292 ASVs); however, no significant difference was observed in the Shannon, Simpson, and Pielou indices. Taxonomic assignment at the genus level showed a total of 157 and 60 genera, respectively. In "Salada Grande" was possible to identify fungal taxa previously reported as halotolerant, such as *Cladosporium*, *Coprinopsis*, *Monosporascus*, *Neocamarosporium*, *Periconia*, and *Sporormiella*, which were not identified in the freshwater lagoon. These results expand our understanding of the diversity of fungal communities in the saline lagoons in Europe, as well as the possible potential for the identification of new fungi in these ecosystems.

New species of chaophilic fungus isolated in hypersaline sediments of Salar de Uyuni

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Salar de Uyuni (SdU) is located in the Bolivian Altiplano at 3650 m above sea level and is considered the largest lithium reserve in the world, hence it is of great environmental and technological interest. SdU is the largest hypersaline environment of the atalassohaline type on the Earth, characterised by its chaotropic chemical composition. Chaotropicity is a chemical activity of some molecules such as $MgCl_2$ or $LiCl$, abundant in SdU, which affect the hydrogen bonds influencing the order of the structure of the biomolecules. Conversely, kosmotropicity is the chemical property of some molecules such as $NaCl$, also highly abundant in SdU, which promotes the formation of these intramolecular bonds. A concentration gradient of salts was observed in SdU, where the concentration of $NaCl$ is higher in the north of the salar (non-industrial area) and the concentrations of $MgCl_2$ and $LiCl$ are higher in the south (industrial area). Likewise, water activity (a_w) levels below the permissible limit for life were observed. A fungus was isolated in an oligotrophic 20% $NaCl$ culture medium from a sediment sample taken from the industrial area. Molecular identification determined that the fungus Zi4_R2A20_208 is a new species. Morphological characterisation and sequencing of different genes (ITS, LSU, SSU, *tef1*, *RPB2*, *tub2*) supported its accurate description. Furthermore, growth on chaotropic salts present in SdU was characterised. The fungus exhibited a tolerance to high concentrations of both chaotropic and kosmotropic agents. Similarly, the fungal isolate showed high levels of tolerance to high concentrations of different heavy metals (As^{5+} , Cd^{2+} , Cu^{2+} , Ni^{2+}).

Shoreline Soil Fungal Microbiomes at Great Salt Lake, Utah

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The saline soils comprising the shoreline of Great Salt Lake, Utah (GSL) provide a unique habitat for fungi and a select few halophytes (salt-tolerant plants). However, until recently, little has been known about the fungal diversity of these soils. Here we present the results of a four-year study characterizing the microbiome of both plant-free bare soil and that of the halophyte rhizosphere at two GSL locations; the more saline North Arm near the artwork, Robert Smithson's Spiral Jetty (SJ), and the less saline South Arm on Antelope Island (AI). Plant-free bare soil and the rhizosphere of *Salicornia utahensis* (Pickleweed) and *Suaeda erecta* (Seepweed) located along the shoreline at both SJ and AI locations were sampled over a four-year period. For each sample, soil salinity was quantified, and DNA was isolated. Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) long read 5k ITS1-4 diversity assay was used for the identification of fungi. Our data suggest significant differences in the composition of fungal species in plant-free bare soil and rhizosphere microbiomes of *Salicornia* and *Sueda* depending on location and soil salinity. In general, decreased diversity of fungal species was observed in both plant-free bare soil and both rhizospheres at SJ compared to AI. Interestingly, a corresponding increase in the representation of halophilic fungi at SJ was observed, possibly linked to the much higher salt concentration in the North Arm. Our results provide insight into the fungal diversity of GSL shoreline soils, whether plant-free or in the rhizosphere of halophytes, and seek to expand our knowledge of halophyte-halophile relationships.

Lignocellulosic degradation on hypersaline conditions by moderate halophile *A. sydowii*

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The use of plant biomass as feedstock for biomaterial and biofuel production is relevant in the current bio-based economy scenario of valorizing renewable resources. Fungi are between the microorganisms that can degrade complex and recalcitrant plant polymers through the secretion of different enzymes that hydrolyze the cell wall polysaccharides. *Aspergillus sydowii* is a moderate halophile isolated from a sugarcane bagasse fermentation supplemented with 2M NaCl, that can grow on different lignocellulosic substrates such as wheat straw, agave fibers, among others, producing cellulases, xylanases, peroxidases and esterases. However, there is a lack of information about the potential of its lignocellulolytic enzymes to deconstruct lignocellulose in salty conditions and the genes encoding the enzymes. This work presents the transcriptomic analysis of *A. sydowii* growing on a semi-solid fermentation of wheat straw with different concentrations of salt (without salt or 0.5 or 2M of NaCl). We analyzed the transcriptome at day 7 of fermentation according to the lignocellulolytic activity results. We found at least 303 genes belonging to the group of glycosyl hydrolases (GH) known as CAZymes in the transcriptome of *A. sydowii*. We identified 39 families of glycosyl-hydrolases (GH), with a particular increase in GH 3, 2, 18, 31, 43 and 92 families. GH3 family corresponds to enzymes with β -glycosidase or 1,4- β -xylosidase activity, whereas those of GH43 family are mainly β -xylosidases and xylanases. Finally, *A. sydowii* expressed a greater number of lignocellulolytic enzymes when growing at 0.5M NaCl, its optimal condition, whereas in non-optimal growth conditions (no salt and 2M of salt) the expression of these enzymes decreases. However according to the enzymatic activity, even though there is a downregulation of CAZy enzymes, there still presence of activity of most of lignocellulolytic enzymes. These results point to the fungus biotechnological potential to degrade lignocellulosic materials in salty conditions, indicating that the lignocellulolytic arsenal is at least halotolerant.

Pigments from halophilic filamentous fungi isolated from saline soils

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Halophiles are a valuable source of unique biomolecules such as extremozymes, biosurfactants, and pigments. Pigments are particularly important as they play a vital role in life on Earth. While synthetic dyes dominate the market, they are harmful to both the environment and public health. Natural pigments, on the other hand, are biodegradable, environmentally friendly, and have biological benefits such as antioxidants and anticancer properties. Fungal species are known for producing pigments with a wide range of colors and multifaceted biological activities. However, the biotechnological applications of pigments from halophilic fungi are less studied compared to those from halophilic bacteria and microalgae. Increasing the use of natural pigments in products can respond to the growing demand for less toxic and more natural materials.

The research aimed to characterize pigments obtained from halophilic filamentous fungi isolated from the saline soil. It was found that certain strains of *Aspergillus* exhibited the ability to produce pigments in a variety of colors, including brown and yellow. Pigments were extracted using various solvents, identified using the UHPLC-DAD-ESI-MS/MS method, and evaluated for their antioxidant properties.

The pigments obtained from one of the tested strains - *Aspergillus niger* FF14 were identified as xanthophylls, with the presence of isomers of violaxanthin, antheraxanthin, and neoxanthin. These carotenoids have not yet been found in halophilic fungi. This is a significant finding, as it expands our knowledge of the diversity of pigments present in these types of fungi and may have implications for the use of these organisms in various applications, such as food production or biotechnology.

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Metabolomic study of a halotolerant black yeast: *Hortaea werneckii*

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Hortaea werneckii is a halotolerant black yeast from the Teratosphaeriaceae family. It is pathogenic for humans, causing ‘Tinea nigra’, a superficial fungal infection, and also responsible for occasional marine fish diseases. ^[1,2] This yeast is distributed primarily in hypersaline, marine and in terrestrial environments, from Atacama Desert rocks to deep sea water. ^[3,4] While some biosynthetic gene clusters seem to be present in this yeast, ^[5,6] very few chemical investigations have been carried out, making *H. werneckii* an interesting candidate for marine biodecovery.

The aim of this work is to describe the chemical diversity of 64 different strains of *H. werneckii*, both haploid and diploid, isolated from multiple environments.^[3] A metabolomic study was therefore conducted to compare their composition and highlight some interesting chemical entities, and also to identify the possible relationships between metabolomes and genomes of the different strains. The strains were cultured on MEA with and without NaCl, and a total of 398 lyophilized samples (3 replicates in each condition) were extracted with ethyl acetate. The extracts were then analysed through UHPLC-HRMS/MS and the data were treated with MZmine software and subjected to statistical analysis. A Feature-Based Molecular Network was then elaborated on GNPS. Furthermore, the results will be compared with the genomic profiles. This will help to understand the relationship between the chemodiversity of the strains and their biology and physiological environment.

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Structure and functions of the fungal microbiome of *Festuca pruinosa*, a chasmophytic and halophytic grass

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Festuca rubra subsp. *pruinosa* (= *Festuca pruinosa*) is a perennial grass which grows in rocky sea cliffs. This is an inhospitable habitat where soil and nutrients are very limited and exposure to salinity and wind is intense. *Festuca* plants count with some structural and biochemical characteristics useful to adapt to this environment. However, the possible contribution of symbiotic mechanisms is unknown. In a survey of the endophytic microbiome of *F. pruinosa* roots, about 135 culturable fungal species were identified. *Fusarium oxysporum* and *Diaporthe atlantica* were among the components of the core microbiome. In addition, the prevalence of *Epichloë festucae* was high in leaf tissues. *Festuca* plants artificially inoculated with *Fusarium oxysporum* and other root endophytes showed increased biomass and salinity tolerance. Similarly, agronomic species such as tritordeum, perennial ryegrass or tomato inoculated with *Diaporthe* endophytes from *Festuca* showed improved growth and tolerance to osmotic stress. Fungal modulation of plant mechanisms involved in osmotic stress management and growth, coupled with fungal roles in organic nutrient recycling might be responsible for the beneficial effects observed in symbiotic plants.

Xerophilic fungi contaminate cultural heritage objects on display indoors

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The study of fungal infestation of cultural heritage objects is gaining interest, in part to understand the mechanisms of fungal growth and the changes they cause to the materials and integrity of the objects. However, current standards for studying fungal growth on objects stored indoors, such as canvas paintings, generally use standard medical mycology culture media (e.g., Sabouraud dextrose agar). As a result, the most important active mycoflora under conditions of low water activity (low relative humidity), the xerophilic fungi, are overlooked. This contribution presents the sampling and cultivation targeting xerophilic fungi in comparison to strategies and results obtained with classical culture media and conditions for mesophilic communities. Culture-dependent methods are complemented by culture-independent ones, which can also be influenced by sampling strategies developed for non-xerophilic communities. Biodeterioration tests applied to xerophilic fungi, such as cellulase, caseinase, esterase, and pigment dissolution assays are presented.

The most important taxa, currently not recognised by restoration mycologists, contaminating indoor cultural heritage objects such as paintings, include xerophilic *Aspergillus* (*Asp.* section *Restricti*), such as *A. vitricola*, *A. magnivesiculatus*, *A. tardicrescenc*, *A. destruens*, and xerophilic *Wallemia* species, such as *W. muriae* and *W. canadensis*.

Cultivation strategies from cultural heritage assets are of great importance because the species name provides an important clue to the information on its biodegradation potency. Isolates of these fungi can be used to test antifungal treatments and to study the biodegradability of recognized as well as new, modern restoration materials. Last but not least, their potential for health problems in restorers dealing with mouldy objects should be investigated.

Desiccation induced extremotolerance: a preliminary study

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Life is based on water as universal solvent; therefore water limitation can be challenging in both aquatic and terrestrial environments. Low water activity (a_w) can lead to the desiccation of cells, halting basal metabolism. Although some studies have shown that desiccated cells can show increased temperature tolerance, chemical and physical stress resistance and diminished consequences of ROS effects, the underlying mechanisms are poorly investigated and understood. In the present preliminary study, black yeast *Aureobasidium pullulans*, isolate EXF-150, was used to investigate whether desiccation could increase resistance to different stresses, particularly high and low temperatures and salinity. Tolerance to high and low temperatures was tested on YNB without NaCl and in the presence of 15% NaCl, respectively, before and after desiccation.

With respect to low temperature, survival rate of desiccated cells was improved both on YNB and YNB+15%NaCl. A different response was obtained for high temperatures. Desiccation for cells grown on YNB increased temperature tolerance for 5°C, from 45°C to 50°C and from 50°C to 55° in 5 min treatments. When cells were grown on 15%NaCl, tolerance to high temperatures diminished for 5°C, from 50° to 45°C after 5 minutes and from 45°C to 40°C after 60 min treatment. These preliminary results represent a promising starting point encouraging further studies that will lead to a better understanding of the response of desiccated cells to environmental stresses.

Primary evidence of new species for *Penicillium* Section *Chrysogena* colonizing halites at Salar Grande, Atacama Desert

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Penicillium species classified in section *Chrysogena* are mainly soil-borne fungi; members of this section have received much attention because of their involvement in penicillin production and as contaminants of indoor environments, and food and feed products. Current *Penicillium* taxonomy has introduced the series as another infrageneric classification, and five of them belong to the section *Chrysogena*: *Aethiopicum*, *Chrysogena*, *Crustacea*, *Goetzium* and *Persicina*. The series *Chrysogena* includes twelve accepted species. In our study, four *Penicillium* isolates were obtained from the lithobiontic microbial community colonizing halite rocks at Salar Grande, Atacama Desert, they were subjected to a morphological and molecular characterization. The morphological study was conducted in potato dextrose, oatmeal, and Dichloran Glycerol agar plates. The molecular analysis included the amplification and sequencing of three phylogenetic markers (*BenA*, *CaM* and ITS). Phylogenetically, all four strains form a distinct clade close to *P. chrysogena* and *P. rubens* species. Amplification and sequencing of the *rpb2* phylogenetic marker plus additional phenotypic characterization are needed to confirm and propose that *Penicillium* isolates from Atacama are new species belonging to the section *Chrysogena* series *Chrysogena*.

Session 3 - Fungi from Polar and Alpine Environments

Metagenomes untangle diversity and adaptation of Antarctic lichen-dominated endolithic communities

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Cryptoendolithic communities dominated by lichenized fungi are microbial ecosystems dwelling inside porous rocks that are able to persist at the edge of the biological potential for life in the ice-free areas of the Antarctic desert. These regions include the McMurdo Dry Valleys, often accounted as the closest terrestrial counterpart of the Martian environment and thought to be devoid of life until the discovery of these cryptic life-forms. Despite their interest as a model for the early colonization by living organisms of terrestrial ecosystems, little is known about the evolution, diversity, and genetic makeup of microbial species that reside in these environments and about the importance of inter-kingdom interactions that improve the community fitness. To address this knowledge gap, we generated the first metagenomes from 109 rocks collected in Continental Antarctica over a distance of about 350 km along an altitudinal transect from 834 up to 3100 m above sea level (a.s.l.). We generated 4,539 metagenome-assembled bacteria genomes, 49.3% of which were novel candidate species and presented evidence that trace gas oxidation and atmospheric chemosynthesis may be the prevalent strategies supporting metabolic activity. We also retrieved a viral catalog comprising > 75,000 viral operational taxonomic units and predicted auxiliary metabolic genes (AMGs) with functions indicating that they may be potentially influencing bacterial adaptation and biogeochemistry. Finally, we assembled 266 eukaryotic genomes, of which 230 were fungi dominated by Ascomycota, mostly representing new fungal candidate species and Tremellomycetes (Basidiomycota). To date, this resource represents the largest effort to date to capture the breadth of microbial genomic diversity from Antarctic rocks and expands the known phylogenetic diversity of inhabiting taxa, focusing on key tolerance competences that promote their spreading and perpetuation at the fringe for life sustainability.

Fungi from polar and alpine environments also colonise household refrigerators

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The Arctic is an environment not only of extremely low temperatures, but also of osmotic stress, high risk of freezing, high UV radiation at the surface, occasional high salinity, and water and nutrient deficiencies. It is now known that extremophilic microorganisms, including psychrophilic and psychrotolerant fungi, live in Arctic environments. In particular, ascomycete filamentous fungi and ascomycete and basidiomycete yeasts of the genera *Candida*, *Cryptococcus*, *Vishniacozyma*, *Naganishia*, and *Leucosporidium* have been isolated from the soil. Numerous ascomycete and basidiomycete filamentous fungi and yeasts of the genera *Cryptococcus*, *Vishniacozyma*, *Naganishia*, *Rhodotoula*, *Leucosporidium*, *Rhodospiridium*, and *Sporobolomyces*, melanized fungi (mainly from the genera *Cladosporium* and *Aureobasidium*), and the genus *Penicillium* have been isolated from aquatic habitats of polar regions (seawater, snow, sea and glacial ice, and cryoconite basins). Cold environments exist not only in nature, but also in our homes, in refrigerators. Microbiologically, the septic tank is the most contaminated, followed by glass and plastic floors and rubber seals of refrigerator and freezer doors. Less colonized are the walls and doors of the refrigerator. Several species of the filamentous genera *Cladosporium*, *Penicillium*, and *Aspergillus* were isolated from household refrigerators. Among the yeast genera, ascomycetes and basidiomycetes from the genera *Candida*, *Debaryomyces*, *Naganishia*, *Cryptococcus*, *Vishniacozyma*, and *Dioszegia* predominated, as well as melanized black yeasts from the genera *Exophiala* and *Aureobasidium*. A comparison of fungal isolates from the Arctic with fungi from a household refrigerator showed an overlap of communities of more than 50%. Thus, the cold environment promotes the preservation of the same fungal species regardless of latitude or habitat origin.

ITS sequence analysis of rock-inhabiting fungi isolated from James Ross Island, Antarctica

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Antarctica offers a harsh and unwelcoming environment ideal for studying extremophilic microorganisms. The diversity, taxonomy, and phylogenetic relationships of fungal communities inhabiting rocks of this unique polar region are being investigated in the frame of the Masaryk University polar research program. The investigated area is situated at the northern deglaciated part of the Ulu Peninsula on James Ross Island, where the Johann Gregor Mendel Czech Antarctic Station is located. By sequencing the ITS rDNA region, 51 rock-inhabiting fungi isolated from rock fragments were examined and compared. The strains were assigned into *Dothideomycetes* and *Eurotiomycetes* classes of the phylum *Ascomycota*. Specifically, 41 strains were placed in the families *Teratosphaeriaceae* (n=20) and *Extremaceae* (n=21), both belonging to the order *Mycosphaerellales* within the class *Dothideomycetes*. Remaining *Dothideomycetes* strains were found to be closely related to species from the orders *Cladosporiales* and *Dothideales*. Four strains showed affiliation with the order *Chaetothyriales*, belonging to the class *Eurotiomycetes*. Overall, 29 analysed strains were classified into four genera of typical rock-inhabiting fungi, namely *Oleoguttula*, *Rachicladosporium*, *Elasticomyces*, and *Vermiconidia*. Among these, the genus *Vermiconidia* stood out with the highest number of isolates, indicating that it represents a common component of the fungal community in the investigated region. Only seven out of 51 analysed isolates were identified to the species *Rachicladosporium antarcticum* (n=2), *Oleoguttula mirabilis* (n=4), and *Elasticomyces elasticus* (n=1). Additional investigation and characterization of the majority of analysed strains are necessary to determine their taxonomic affiliation accurately and for a description of potentially new taxa. Melanised microscopic fungi inhabiting the rocks of James Ross Island constitute a polyphyletic group with a notable prevalence of members from the class *Dothideomycetes*. These results contribute to the understanding of fungal diversity in this region of Antarctica and indicate the existence of unexplored fungal taxa.

Chytridiomycetous fungi are major controls of glacier ice algae biomass on the Greenland Ice Sheet

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Ancylonema alaskanum and *A. nordenskioldii* dominate glacier ice algal communities on the margins of the Greenland Ice Sheet and play important roles in the darkening and melting of its surface ice layer. While culturing and field characterization efforts have begun to elucidate the life cycle, nutrient requirements and photophysiology of these glacier ice algae, little is known about their top-down controls. Previously, we confirmed the presence of chytrids as the dominant fungi in various habitats on the Greenland Ice Sheet by amplicon sequencing. These zoosporic fungi of the Chytridiomycota phylum are recognized as lethal parasites of algae in Arctic freshwater and marine environments. Further, recent studies and *in-situ* observations identified the presence of undescribed chytrids infecting *Ancylonema* sp. blooms, raising awareness on their importance and questioning the ecological role of these parasites in the dynamics of ice algal blooms. Here, we present the first trials to grow parasitic chytrids in co-culture using *Ancylonema* sp. as the host and the first quantitative assessment, using qPCR and total RNA approaches, of how the zoosporic fungi performed throughout two summer seasons on the Greenland Ice Sheet. Staining with the vital dye FUN-1 revealed metabolic activity of the encysted zoospores and provided insights into the developmental stages of chytrid sporangium.

Session 4 - Astrobiology and Fungal Dispersal

Exploring filamentous fungi biofilms under simulated microgravity on analog Martian and Lunar regoliths

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As crewed exploration of the Moon and Mars is becoming more likely in the 21st century, research efforts continue to be directed toward creating and optimizing bioregenerative life-support systems (BLSS) and ways to produce food, medicine and other resources outside of Earth's conditions. Since microorganisms have been found in all major past and present human-made space structures, they will undoubtedly be included in future lunar and Martian exploration, not just as passive companions, but as active components of BLSS and facilitators of *in situ* resource utilization (ISRU). One interesting aspect of relevant microorganisms such as filamentous fungi is their ability to form biofilms. Biofilms can damage the surface they form on, and often exhibit an increased antifungal resistance and virulence compared to regular cultures. Therefore, if actively included in space exploration, they pose a risk to both structures and astronauts. While biofilm formation has been characterized to some extent under Earth conditions, significantly less is known about how fungal biofilms behave under extra-terrestrial conditions.

In an attempt to identify where future studies should put their focus, we conducted a study on the influence of two environmental aspects on biofilm formation: 1) growth under simulated microgravity and 2) growth in the presence of regolith as a substrate. We grew fungi on planetary regolith simulant platforms (LHS-1, LMS-1, JEZ-1, MGS-1, Exolith Lab, USA) both under Earth-gravity and under simulated reduced gravity in a 3-D clinostat (Gravite, As One International, Japan), with the aim to identify whether differential gravity or substrate play a stronger role in biofilm formation, and overall whether biofilm formation under these conditions is possible.

Screening of extremotolerant filamentous fungi for the biogenic synthesis of metal nanoparticles under simulated microgravity

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Metal nanoparticles (MNPs) have gained increasing attention due to their wide variety of applications as antimicrobial agents, drug carriers, additives, in several fields, like pharmaceutical, water treatment, agricultural, food industry, among others.

Despite chemical and physical methods being used for their production, microbial-mediated synthesis processes are preferable as they are seen as eco-friendly. Mycogenic synthesis of MNPs from filamentous fungi has been reported by a number of studies where cultivation conditions seem to be crucial in determining sizes, shapes and other properties of the particles. In line with this, the constant search for new products has shifted the attention to extremophilic and extremotolerant species as potential producers of compounds with novel and unusual characteristics and thus, as possible eco-friendly nano-factories.

Reflecting this tendency, the objective of the present study was to screen extremotolerant species of filamentous fungi for production of titanium dioxide nanoparticles (TiO₂NPs), and silver nanoparticles (AgNPs) under different settings, including simulated microgravity conditions, as an example of a stress condition for growth. Indeed, microgravity is a factor known to induce various changes in organisms, including fungi, by affecting the distribution of body fluids as well as through other as-yet-unidentified mechanisms. Production of MNPs was detected in most of the strains screened, however the resultant MNPs presented differences in their characteristics when exposed to stress. Our results demonstrate that MNPs production by fungi is affected by exposure to microgravity, and the differences in the MNPs produced with and without exposure are species and strain dependent.

Session 5 – Interactions

Lichen mycodiversity in Antarctica reveal the key role of thallus structure as suitable niche for diversity

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Lichens are complex symbiotic organisms consisting of a main fungal partner (the mycobiont), a population of main photosynthetic partners (the photobionts), and a heterogeneous array of microorganisms, which includes other microfungi (lichenicolous fungi), microalgae and bacteria. Recent studies have reported very complex structures of lichenicolous fungal communities inhabiting lichen thalli (the lichen mycobiomes) and have shown that their diversity correlates with the lichen host species and the ecological conditions. Most representatives of the lichen mycobiomes are extremophilic fungi known for their polyextremotolerant traits. So far, the mycodiversity of endemic Antarctic lichens has not been investigated and has never been compared with both the endolithic fungal communities known from the Antarctic environment and cosmopolitan lichens. To address this gap, we conducted a study on five endemic (*Acarospora flavocordia*, *Buellia frigida*, *Lecanora fuscobrunnea*, *Lecanora physciella*, *Lecidea cancriformis*) and three cosmopolitan (*Pleopsidium chlorophanum*, *Rhizoplaca melanophthalma*, *Rusavskia elegans*) lichen species collected in the Antarctic region of Victoria Land. Our aims were to: i) investigate the mycodiversity associated to endemic and cosmopolitan lichen species in Antarctica, ii) correlate the diversity of the lichen mycodiversity to that of the endolithic fungal communities of Antarctica. We approached these tasks by performing DNA metabarcoding analyses and culture isolations of micodiversity associated to lichens. We found a high diversity of lichen-associated fungi in both endemic and cosmopolitan lichen species. Alpha and beta diversity patterns of the lichen mycodiversity revealed differed fungal composition in endemic and cosmopolitan species, with most of the ASVs being still unknown taxa. 200 isolated strains were genetically and morphologically characterized and highlighted the presence of already known lichen-associated fungi as well as new lineages of extremophilic taxa represented both by Ascomycota and Basidiomycota.

A lichen-associated black fungus distributed across continents, climate zones, and host species

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‘Black fungi’, or ‘black yeasts’, are commonly associated with the lichen symbiosis. Whether these fungi are coincidental inhabitants, parasites, commensals, or integral parts of the mutualism, is currently little understood. Black fungi can be isolated from lichen thalli and grown in culture. Here, we report the finding of a particularly widespread black fungus, belonging to the Chaetothyriales (Eurotiomycetes). We isolated cultures of this fungus from several lichen thalli, and designed taxon-specific ITS primers to search for the species in DNA extracts from diverse lichen specimens. We recovered ITS sequences of this specific black fungal lineage from two continents (North America and Europe), different climate zones in each continent (cold temperate and Mediterranean), and different lichen host species (e.g., *Umbilicaria phaea*, *U. pustulata*, *U. freyi*, *U. polyphylla*, *Dermatocarpon miniatum*, *Pseudevernia furfuracea*). This indicates that the fungus has few, if any, dispersal limitations, tolerates a broad range of climatic conditions, and can live in association with a variety of unrelated lichen-forming fungi. GenBank BLAST searches yield identical ITS sequences belonging to fungi, which have been isolated from rock surfaces, implying that the fungus is probably not confined to the lichen symbiosis, but also occurs as a free-living species. We are currently developing genomic resources for this interesting species to pave the way for population genomic and phylogenomic studies. Evaluating genome content and genomic features will provide clues to better understand the fungus’ lifestyle and ecological versatility.

Session 6 - Radiation, Acidity and Other Extreme Environments

Characterisation of selected acidophilic and alkaliphilic fungi

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Fungi are morphologically and ecologically plastic microorganisms, highly adapted to life in diverse extreme environments. Many species are known by their ability to grow in a wide range of physico-chemical factors, one of such is also a wide pH range. In our work, we hypothesize that fungi isolated from extreme environments are able to adapt, survive and possibly actively grow in a wide range of pH scale. Experiment includes fungi isolated from the concrete of the Plečnik monuments, which has a pH between 8 and 11, and fungi isolated from hot acidic lake in the crater of the Chichonal volcano in Mexico, with pH between 2 and 4.

To determine fungal acidic and alkali potential and their need for oxygen, we incubated the selected strains for 7 days in a liquid medium with pH 7. *Acidomyces acidothermus*, *Aureobasidium sp.*, *Cladosporium allicinum*, *Botrytis pelargoni*, *Parengiodontium sp.*, strains were characterized as facultative anaerobes, growing through the whole medium column, while *Trametes versicolor*, *Penicillium chrysogenum*, *Penicillium janthinellum*, *Aspergillus pseudoviridinutans* were strictly aerobic, observed only at the top of the medium. *Parengiodontium sp.*, *A. acidothermus*, *P. janthinellum*, *C. allicinum*, *Rodothorula taiwanesis*, *P. chrysogenum*, *T. versicolor*, *Penicillium bialowiezense*, *B. pelargoni* strains lowered the pH in a range from 6.86 to 3.74, with the species *P. chrysogenum* having the biggest acidifying potential. *C. allicinum* did not change the pH, and *A. pseudoviridinutans* slightly increased pH.

To observe fungal growth in a pH gradient from 2.3 to 12.5 we incubated strains for 14 days in a liquid medium containing Tween 80 and peptone with buffers adjusted pH. Any changes in pH of the medium have been monitored before inoculation and at the end of the experiment, compared to the negative controls (medium without inoculum) to assess the possibility of fungi to lower or increase the pH of the initial environment. In addition, we microscopically visualized phenotypic changes in fungi growing at their pH limits.

Carotenoids protect *Rhodotorula mucilaginosa* against oxidative stress

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Rhodotorula sp. yeasts survive adverse situations such as high salt concentration, UV radiation, low temperature, and heavy metal contamination. *Rhodotorula* yeasts produce carotenoids and most research has been focused on increasing the synthesis of these compounds using different substrates or stressors. The physiological role of carotenoids in this yeast is not clear. The role of carotenoids in *Rhodotorula mucilaginosa* physiology was evaluated. Diphenylamine (DPA) was used to inhibit carotenoid synthesis. Oxidative stress was produced by menadione addition. Also, the role of carotenoids in fermentative (YPD, dextrose) and non-fermentative (YPLac, lactate) conditions was evaluated. *R. mucilaginosa* generated more biomass in YPD (2.1-fold) than in YPLac. In contrast, carotenoid concentration was higher in YPLac than in YPD. In addition, carotenoids reached the highest levels late, at the stat phase. To determine whether carotenoids quenched reactive oxygen species (ROS), hydrogen peroxide was measured in DPA yeast treated. In YPD cells, addition of 40 μM DPA increased H_2O_2 2.5 fold, while in YPLac, 40 μM DPA increased H_2O_2 6.3 fold. Further experiments performing dilution spot assays at different DPA concentrations to decrease carotenoid production and in the presence of two different menadione concentrations demonstrated that YPLac cells were more susceptible to ROS than YPD cells. It is suggested that in *Rhodotorula mucilaginosa* carotenoids protect cells against both, endogenous and exogenous oxidative stress.

The use of fungi in concrete repair

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Concrete is the most used man-made material in the world. As a building material, it is characterized by solid compressive strength and durability, ease of installation and accessibility, and chemical reactivity on the one hand, and by fragility, low tensile strength and resulting cracks on the other hand. These occur due to everyday (mechano-)physical, chemical, and biochemical factors, reducing the durability of concrete and accelerating its deterioration, by facilitating transports and chemical reactions in the microstructure of the material. Therefore, preservation of its initial (unfissured) state and maintenance of its mechano-physical properties, mostly with recovery measures are key to maintain its durability. Fungi capable of biomineralizing calcium minerals that are highly compatible with concrete are candidates for its restoration and could overcome or surpass the deficiencies of bacterial regeneration through biomineralization. Fungi form biominerals either by induced or organo-mineralization. Due to high alkalinity of concrete (pH about 13 in fresh state which during carbonation processes reduces to a value less than 9), alkali-tolerant or alkaliphilic fungi are desirable for recovery purposes. Aim of this study was to identify fungi suitable for concrete healing. A selection of strains preserved in the collection of extremophilic fungi Ex isolated from stone, concrete, or belonging to species otherwise known for biomineralization, was evaluated for their ureolytic activity, formation of biominerals during the metabolism of urea with the addition of calcium (Ca^{2+}) ions (CaCl_2 , $\text{Ca}(\text{NO}_3)_2$), and for their tolerance to high pH. The best candidates were grown on cement pastes (cured 28 days, non-carbonated and exposed to accelerated carbonation) with known pH value and evaluated for their concrete healing potential.

Oligotrophic water systems as reservoirs for selection of fungi in drinking water

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Safe drinking water is a constant challenge due to global changes and the rise of emerging pathogens. According to the latest data from the WHO, these include also fungi. Although their presence in water is well documented, selection of fungi on different materials are scarce. *In vitro* testing has shown that spores and pigmented fungal cells can survive disinfection and could later form biofilms in water network systems. However, due to the lack of uniform sampling and identification methods, fungi are not yet considered as a microbiological parameter in the European Drinking Water Directive. During the sampling of water at various stages of water treatment in Slovenia we isolated on the average 260 CFU/L from raw water before disinfection, 49 CFU/L from water after ultrafiltration and chlorination, 64 CFU/L at the first sampling point in water network and 97 CFU/L at the last sampling point. The occurrence of fungi in the entire drinking water supply system was mostly influenced by the location of the primary aquifer. Chlorine reduced the number of fungi in drinking water on the average by 5-times, but its effect decreased with the distance of water network. Thus, at the final sampling points, we detected on the average 2-times higher numbers of fungi compared to freshly chlorinated water. Compared to water, we noted positive selection of different fungi on materials. The presence of the genera *Aspergillus*, *Acremonium*, *Furcasterigmium*, *Gliomastix* and *Sarocladium* was most common on cement, while *Cadophora*, *Cladosporium*, *Cyphellophora* and *Exophiala* prevailed on metals. Plastic materials were more prone to colonization with basidiomycetous fungi. Opportunistically pathogenic fungi of the genera *Aspergillus*, *Candida*, *Filobasidium* and *Exophiala* were isolated sporadically from both, materials and water. All tested *Aspergillus* spp. strains were susceptible against nine antimycotics, but *Candida parapsilosis* showed intermediate resistance to anidulafungin, micafungin, caspofungin and flucytosine.

Fungi subsisting in aged resin-contaminated soil

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Resin is produced by conifers as a defense mechanism against injury and microbial infections. Resin's antimicrobial properties are mostly due to diterpenoid resin acids, which are toxic to many microorganisms. However, some fungi have the ability to dwell in resin. Most studies have focused on plant-pathogen interactions or the biotransformation ability of generalist fungi; only a few have investigated fungi specifically growing in resin. This suggests that resinous environments should be studied as natural habitats for fungi, as well as the effects of resin as a natural stressor and modulator on fungal communities, which remains partially unknown.

Our study focuses on whether long-term exposure of a fungal community to resin can lead to the emergence of specialized resin-degrading microorganisms. Here, we specifically analyzed an aged resin-contaminated soil collected from an abandoned resin refinery (closed in 1970s), which has remained undisturbed (Portugal). The mycobiota was analyzed using high-throughput sequencing of environmental DNA and taxonomic identification of fungal isolates (ITS marker). Complementary soil analysis focused its physiochemical properties and presence of resin acids (nuclear magnetic resonance, NMR). The soil shows a C/N signature altered by the presence of an aged resin. The fungal community composition displays an unusual diversity, including potential resinicolous fungi. The ITS sequences of the 170 fungal isolates were compared to the amplicon sequencing data to determine their position in the fungal community. Preliminary data revealed new fungal species (under validation). Some isolated strains were previously associated with hydrocarbon-degrading abilities, for example *Amorphoteca resinae* and *Candida keroseneae*.

This study shows that the aged resin-contaminated soil hosts a unique mycobiota, enriched in specialized hydrocarbon and resin-degrading strains.

Effect of selected drying programmes in a commercial heat pump tumble dryer for household use on the microbial load of laundry

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Energy and water-consumption constraints have changed the laundering habits of washing machine users, who often use eco programmes with low/medium-temperatures (30-60 °C) and biodegradable liquid detergents without oxidising agents. Such washing practices may remove stains and dirt from soiled textiles, but often do not provide adequate cleaning and disinfection of the laundry. This can ultimately favor the growth and transmission of odour-causing microorganisms and opportunistic pathogens, posing a risk to human health. However, subsequent drying may contribute to additional inactivation of the microbial contaminants. In this study, we aimed to evaluate the antimicrobial efficacy of three drying programmes - two standard programmes and one dedicated anti-allergy programme - of a commercial heat pump tumble dryer for household use with a maximum load capacity of 8 kg. Antimicrobial efficacy was determined according to a standard procedure using biomonitors, i.e. standard cotton swatches artificially contaminated with either a calibrated planktonic inoculum of individual test microorganisms, Gram negative (*Escherichia coli*, *Pseudomonas putida*) and Gram positive bacteria (*Enterococcus faecium*, *Micrococcus luteus*, *Staphylococcus aureus*) and yeast (*Candida albicans*) or a multispecies biofilm (consisting of a synergistic four-species bacterial consortium and an opportunistic fungal pathogen *Exophiala dermatitidis*) and dried together with 4 kg of standard cotton ballast laundry with an initial moisture content of 60±1%. In addition, the tumble dryer and ballast laundry were equipped with different sensors to monitor the experimental conditions and evaluate the contribution of the different physical variables to the inactivation rate. It was found that all the drying programmes tested inactivated the microbial load of the biomonitors. The dedicated anti-allergy drying programme was most effective against all microorganisms tested. This programme was most effective against the Gram-negative bacterium *E. coli*, with an average reduction rate of 99.997% (log reduction 4.6). Inactivation of the tested Gram positive bacteria and the yeast *C. albicans*, on the other hand, was lower. We recorded an average reduction rate of 94% (log reduction 1.4) and 97.8% (log reduction 1.7), respectively. However, the microorganisms that established biofilm directly on the biomonitors seemed to be less affected by the drying programmes than the planktonic inoculum of the individual test microorganisms. We can conclude that dedicated anti-allergy programme had a good time-temperature relationship for efficient inactivation of the tested mesophilic microorganisms under the given experimental conditions. Higher temperatures and longer drying, resulting in a much lower moisture residue in the laundry, played an important role in inactivating the exposed microorganisms.

Fungi from high altitude crater lakes and glaciers from Iztaccíhuatl volcano (Mexico) as a source of human opportunistic pathogens and heavy metal remediation

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Extremophilic, cold-adapted fungi exhibit active metabolism in glaciers and ice sheets exposed to extreme conditions. These organisms have adaptations related to the cellular processes to survive in harsh environments. Iztaccíhuatl volcano, Mexico (5,210 masl) hosts two glaciers and two crater lakes, which could be an important source for research on cold-adapted fungi. Characterizing the ability of fungi to survive in these extreme environments could help understand their role in virulence processes in humans, resistance to antibiotics, and tolerance to heavy metals. In the present study, fungal diversity at four sites (two crater lakes and two glaciers) was described using Illumina amplicon sequencing (ITS rDNA). In addition, fungal isolates were cultured in solid media, identified using ITS sequencing, and characterized for pathogenicity traits (hemolysis, thermotolerance, and resistance to antifungal drugs) and tolerance to heavy metals (Cr(III), Pb(II), and Hg(II)). Glacier and water samples were analyzed and results of Illumina ITS fungal sequences showed that Ascomycota, Basidiomycota, and Chytridiomycota were the most abundant phyla. The most abundant classes were Microbotryomycetes, Tremellomycetes, and Dothideomycetes. In the crater lake samples, *Leucosporidium* was the dominant genus, while *Phenoliferia* prevailed in the glacier samples. Phylogenetic identification of cultivated fungi allowed partial identification of 39 isolates at the genus level belonging to the phyla Ascomycota and Basidiomycota. These included primarily the genera *Mrakia*, *Phenoliferia*, and *Naganishia*. All isolated fungi were psychrotolerant, some hemolytic and resistant to various concentrations of antifungal agents (ketoconazole, itraconazole), as well as to heavy metals. Through the combination of high-throughput amplicon sequencing, cultivation and pathogenetic characterization of fungal isolates, we described fungal diversity and demonstrated the potential of extremophilic fungi as opportunistic pathogens for humans. Knowledge of fungal biology in extreme environments, such as Mexican glaciers, should be further explored in light of the current rates of global warming leading to permanent changes.

Dark septate endophytes isolated from wild plants under acidophilic and metalliferous conditions

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Dark septate endophytes (DSE) consist of a very diverse and valuable group of ascomycetes. They are ubiquitous root-colonizing fungi characterised by their highly melanised cell walls and by their septate hyphae. DSE have been described to improve the nutrient uptake and protect plants from different abiotic stresses. They may be the dominant colonisers of plant roots in areas with metalliferous soils since melanin chelates heavy metals by virtue of quinoid groups in its chemical structure. Therefore, DSE may prevent the accumulation of heavy metals into the plant tissue, thus reducing toxicological hazards in edible products. However, DSE are not commonly isolated from the environment because of their rather low competitive ability under laboratory conditions. We have adapted the isolation method known as the 'oil flotation' technique that consists of exploiting the hydrophobicity of melanized fungi. To our knowledge, this method has been applied successfully to plant rootlets for the first time and we have obtained a wide collection of DSE fungi.

In this study, DSE of particular interest were isolated from *Phagnalon saxatile* and *Erica andevalensis* root samples collected from the Odiel and Río Tinto riverbanks, both located in the province of Huelva, Spain. There used to be an intense mining industrial activity in the area, which caused an increase in the content of heavy metals and a strong acidification (pH = 3.07 in Odiel and pH = 1.83 in Río Tinto) of the affected soils and sediments. The content of Cu, Fe, Ni, As, Co, Cd and Zn was analysed for both water and sediments where plants were collected. Isolated DSE were identified and characterized as agents that may be related to the alleviation of environmental stress and to the enhancement of plant adaptation to the toxic effects of heavy metals.

Session 7 - Black Fungi

Is homothallism widespread in the Chaetothyriales?

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Sexual reproduction is a main factor for the generation of genetic diversity, and can affect the evolution and adaptation of an organism. In fungi, the mating locus genes largely determine how sexual reproduction occurs. These loci, which seem to have origin in a single ancestral locus, control whether a haploid fungus will present a homothallic or heterothallic sexual reproduction strategy. While homothallism is associated with higher compatibility, heterothallism results in higher degrees of recombination. In the *Chaetothyriales*, a previous study has identified a homothallic system in only 15% of the species in the order. To further investigate the occurrence of homothallism in *Chaetothyriales*, an order that contains species of clinical and of biotechnological interest, we generated Hidden Markov Model (HMM) probabilistic evolutionary profiles for *MAT1-1* to *MAT1-1-5* locus using GenBank reference fungi sequences previously identified for those loci. The *MAT1-1* (alpha box) profile was accurate at E-values below 1.5×10^{-6} , and *MAT1-2-1* (high mobility group box - HMG - domain) below 9×10^{-36} , but the results for the *MAT1-1-3* to *MAT1-1-5* loci showed a large overlap with the *MAT1-2* locus. Besides the identification of *MAT1-2* in about 30% of the species examined (several in the *bantiana* clade), the phylogenetic analysis of all the loci showed that the relationship between locus *MAT1-1-3* to *MAT1-1-5* and alpha box and HMG loci are not well defined, with some analysis indicating that these three can be more related to *MAT1-2* than to *MAT1-1*. Most of the sequences previously identified as *MAT1-1-4* do not present any of the defining structures found in *MAT1-1* or *MAT1-2*, and alignment of these sequences shows two distinct patterns that can be related to different origins. Given these considerations, is possible that homothallism is more widespread in the *Chaetothyriales* than previously reported, a hypothesis that needs further investigation.

Fungal biofilms on materials: describing and modelling growth of the black fungus *Knufia petricola*

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Fungi that grow as biofilms are associated with clinical settings as well as various cases of material fouling and material damage. Black fungi as biofilm formers have been rarely studied so far. Their conspicuous dark pigmentation, EPS production, adhesion capabilities and adaptations to stresses allow black fungi to develop biofilms on materials under harsh conditions. For example, rock-inhabiting black fungi withstand sun irradiation and dehydration and are therefore ubiquitous on arid surfaces like solar panels and marble monuments.

To understand and control their ability to colonise and deteriorate materials, one should assess and model black fungi's growth patterns. But so far, no mathematical model has been developed to describe their growth. *Knufia petricola* A95, representing rock-inhabiting fungi from Chaetothyriales, is genetically amenable and can serve as a model for biofilm studies in black fungi. The primary objective of this project is to develop a growth model for *K. petricola* A95 which will enable to define and predict material colonisation of black fungi.

Dedicated experimental work with *K. petricola* will allow the quantitative assessment of the impact of environmental conditions (e.g. pH, nutrients, etc.) on the growth behaviour at the biofilm and single cells level. Data which will be used to validate and develop an individual-based model (based on the iDynaMICS modelling platform) that explains how fungal biofilms form, colonise materials, and cause deterioration.

Thus far, research has been conducted on the impact of different concentrations and sources of major elements (e.g. C, N, ...), as well as trace elements (e.g. Cu, Mg, ...), on the colony shape and biomass of *Knufia petricola* A95 biofilms. To study the behaviour of single cells, the length of the cell cycle in different growth media has been determined via the combined use of microfluidic devices and confocal microscopy.

A non-canonical septin in the polyextremotolerant black yeast *Knufia petricola* recapitulates many features of canonical septin octamers

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Morphological complexity and plasticity are hallmarks of polyextremotolerant fungi. Septins are conserved cytoskeletal proteins and key contributors to cell polarity and morphogenesis. They sense membrane curvature, coordinate cell division, and influence diffusion at the plasma membrane. Four septins homologs are conserved from yeasts to humans, the two systems in which septins have been studied most extensively. All are GTP-binding proteins. These canonical septins assemble into palindromic octamers, which can then form higher-order structures such as the collar at the bud neck of budding yeast and bars in the hyphae of filamentous fungi. But there is also a fifth family of septin proteins that remain biochemically mysterious, because they are not found in model fungi. Members of this family, known as Group 5 septins, appear in the genomes of filamentous fungi, ciliates, diatoms, chlorophyte algae and brown algae— a wide distribution suggesting an ancestral septin lost in multiple lineages. Thus, Group 5 septins may offer us a window into the evolutionary history of the septin cytoskeleton, but they can only be studied in non-model or emerging model systems, such as black fungi.

Knufia petricola is an emerging model black fungus, for which CRISPR-based tools have been developed. Through a combination of *in vivo* fluorescence microscopy and *in vitro* biochemical reconstitution, I am characterizing the functions of *Knufia petricola*'s Group 5 septin, AspE. Thus far, I have demonstrated that recombinantly expressed and purified AspE can form diverse homo-oligomers, bind and exchange GTP, and sense membrane curvature *in vitro*. By comparing *Knufia* septins to canonical septins in model fungi, I am building an understanding of how the septin cytoskeleton has evolved to support varied cell shapes, modes of division, and extremophile lifestyles.

DHN melanin – synthesis, regulation, and functions in *Knufia petricola*

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Dihydroxynaphthalene (DHN) melanin is produced by diverse Ascomycetes via slightly differing biosynthetic routes. The polyketide synthases (PKS) release the heptaketide YWA1, the hexaketide AT4HN or the pentaketide T4HN. The first two products are deacetylated by ‘yellowish-green’ hydrolases to T4HN, and T4HN is further converted by a core set of enzymes to DHN. Final polymerization steps are accomplished by multicopper oxidases. DHN melanogenesis is often regulated in a spatial and temporal fashion resulting e.g., in melanized reproduction and survival structures of the foliar plant pathogen *Botrytis cinerea* (Schumacher 2016, *Mol Microbiol*). In contrast, microcolonial black fungi exhibit constitutive melanogenesis. Here, DHN melanin builds a protective layer around all vegetative cells thus contributing to the survival of diverse environmental stresses even without specialized reproduction structures. For studying the regulation and relevance of DHN melanogenesis for tolerance of abiotic and biotic stresses, adhesion to substrates and subsequent damage of colonized surfaces, the rock-inhabiting fungus *Knufia petricola* was chosen as gene functions in this fungus can be studied by CRISPR/Cas9-based genome editing. The putative melanogenic genes were identified in the genome of *K. petricola*, deleted to confirm their involvement in DHN melanogenesis and co-expressed in *Saccharomyces cerevisiae* for reconstruction of the synthesis pathway. Phenotypes of deletion mutants are studied for specifying the functions of DHN melanin in *K. petricola*.

Session 8 – Omics and Molecular Tools for Extremophilic Fungi

Unleashing the potential: Protoplast-Based transformation and CRISPR/Cas9-mediated genome editing in *Aureobasidium pullulans* and *Hortaea werneckii*

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Fungi of the genera *Aureobasidium* and *Hortaea* are remarkable organisms known for their ubiquitous occurrence and ability to thrive in extreme environments. These black yeast-like fungi exhibit intriguing morphological features, and form melanized cells and biofilms, that contribute to their adaptability and survival in harsh conditions. The particular yeast-like fungi have gained industrial importance as they are utilized in the production of valuable compounds.

In our study, we describe the development of a CRISPR/Cas9 plasmid-based genome editing method for *Aureobasidium pullulans* (EXF-150 and EXF-3645) and *Hortaea werneckii* (EXF-15 and EXF-562), using pAMA/ribo and pAMA/tRNA plasmids, encoding Cas9, sgRNA and resistance to the antibiotic hygromycin (*hygR*). The targeted integration of DNA cassette carrying resistance to the antibiotic geneticin (*genR*, G418) and the nucleotide sequence for nuclear/cytosolic green (GFP) or red fluorescent protein (RFP) at the site of Cas9-induced DSB in genes *ura3* (encoding orotidine-5'-phosphate decarboxylase), *ade2* (encoding phosphoribosylaminoimidazole carboxylase) and *leu2* (encoding beta-isopropylmalate dehydrogenase) in the protoplasts of said strains generated auxotrophic strains of *A. pullulans* and *H. werneckii* with dual antibiotic resistance and expressed fluorescent protein. To screen the transformants, we inoculated the strains on a medium without additional amino acids, and performed PCR screening to confirm the insertion of the DNA cassette at the correct location in the genome. As a positive control for transformation, we used a previously established protocol (Noack-Schönmann et al., 2014), for *Knufia petricola* in which we generated a DSB in the genes *pk1* (encoding polyketide synthase 1, required for melanin synthesis) and *phs1* (encoding phytoene synthase 1, required for the synthesis of carotenoids), resulting in strains deficient in either melanin or both melanin and carotenoid synthesis, that formed pink and white colonies. The production of such strains could be important in explaining some phenomena that have remained unclear till now, such as reproduction strategy. Auxotrophic strains may also be important in avoiding the use of antibiotics and further promoting the rising problem of antibiotic resistance in clinically important species. This method could later become an efficient tool for metabolic engineering of strains employed in industrial settings.

Pore forming proteins in extremotolerant or extremophilic fungi

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Fungi growing under extreme conditions can be divided into ubiquitous and polyextremotolerant generalists, or rare, isolated specialists with narrow ecological amplitudes. While generalists can compete with mesophilic species, specialists cannot. Aegerolysins are remarkable proteins that are relatively abundant in fungi and bacteria, but also in some insects, plants, protozoa, and viruses. Despite their occurrence in cells of certain developmental stages and their presence in secretomes, only a few aegerolysins have been studied in detail. To date, (experimental) data have been published on 23 different aegerolysins and their variants from 18 different species belonging to different kingdoms of the tree of life. What is most fascinating is their function. They are involved in various interactions by recognising a molecular receptor in the target organism. The formation of pores in combination with larger, non-aegerolysin-like protein partners is one of the possible responses of the aegerolysin-producing organism in the competitive exclusion of other organisms from the ecological niche. The machine learning approach to protein structure determination provided new insights into the structure (and function) of aegerolysins and their pore-forming partners. Unexpectedly, aegerolysins - recognisable by the tight β -sandwich folding of low-identity protein sequences - dock five different groups of larger partners to (presumably) form pores. In fungi, the phylogenetic distribution of aegerolysins is inconsistent. They are encoded by several genomes of Dikaria species, but not by the genomes of species of Pucciniomycotina, Ustilaginomycotina, Wallemiomycetes, Orbiliomycetes, Xylonomycetes, Saccharomycotina, and Taphrinomycotina. They are also observed in some Mucoromycota genomes, but not in Glomeromycotina. Since extremotolerant and extremophilic fungi have evolved in numerous branches of the fungal tree of life and aegerolysins are unevenly distributed among fungal species, it is of interest to investigate whether or not aegerolysins and their partner proteins contribute to the extreme survival ecology of generalists or specialists.

Optimization of RNA purification from *Aspergillus puulaauensis* and *Penicillium chrysogenum* isolated from Art Restoration Materials Lascaux Acrylic Glue 498 and Regalrez 1094

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Artistic canvas paintings are often attacked by fungi if they are stored at inappropriate conditions. Fungi cause visible, even structural damage to paintings, especially due to their ability to grow at low relative humidity and production of various enzymes and organic acids. Some paintings can be overgrown by fungi relatively soon after conservation-restoration treatment, even after the use of synthetic materials, which are commonly used as consolidants, varnishes, and to enhance the visual effect. Although this problem is widespread, little is known about fungal growth on various synthetic materials or the effects of storage conditions on these materials.

In this study, we tested and optimized the chosen method of RNA purification of fungal isolates from art paintings (xero- and halotolerant *Aspergillus puulaauensis* and *Penicillium chrysogenum*) growing on synthetic materials commonly used in conservation-restoration (Lascaux Acrylic Glue 498, butyl-methacrylate dispersion and Regalrez 1094, hydrocarbon resin). Fungal biomass was obtained by inoculating the liquid YNB medium with 2% (m/v) glucose with a small agar plug from a fresh fungal plate (MEA) and cultured at 25 °C. Growth was confirmed morphologically with a stereomicroscope, by DNA isolation and re-sequencing of selected genetic markers (β -tubulin). After reaching the logarithmic phase, the biomass was transferred into a liquid medium (1 g per 50 ml of medium) consisting of YNB and 3% (m/v) of the synthetic material. All growth methods were performed in biological triplicate experiments. After one week of exposure to the selected synthetic materials, biomass was collected by centrifugation (10 min, 3500 rcf), immediately frozen, and macerated in liquid nitrogen. Immediately after harvest, RNA was purified by the TRIzol method (TRI Reagent). To increase the yield and quality of RNA, we implemented changes in the RNA purification method. Concentration and purity were measured by NanoDrop spectrophotometer, and integrity and purity were checked by formaldehyde agarose gel electrophoresis (1.3% agarose, 5% formaldehyde in 1 × MOPS buffer (20 mM 3-[N-morpholino] propane sulfonic acid, 5 mM sodium acetate, 1 mM EDTA)).

Fungome of the Sečovlje salterns (Slovenia), the northernmost Mediterranean salterns

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Solar salterns are an interesting environment for studies of halophilic and halotolerant microorganisms. The series of basins with different evaporation rates, in which seawater is gradually concentrated to the point of precipitation of table salt or halite, represent the whole range of salinity from 3% to saturation at about 35% (w/v). After the NaCl is harvested, the salt pans remain with the bitterns. The aim of our study was to describe the fungal diversity in brines of different concentrations and in bitterns from the Sečovlje salterns (Slovenia). We used a combination of metagenomics (amplicon sequencing) and culture-based methods to study the saltern fungi.

The majority of fungal isolates from the brine and bittern of the Sečovlje salterns belonged to the Ascomycota. The most abundant orders were Capnodiales with typical halophilic/halotolerant representatives of the genera *Cladosporium*, *Hortaea* and *Phaeotheca*, and the order Eurotiales with the genera *Penicillium* and *Aspergillus*. Non-melanized Ascomycota yeasts of the genera *Debaryomyces*, *Candida*, and *Meyerozyma* were also present in brine and bittern. Other filamentous fungi such as *Fusarium* and *Alternaria* were also common in the brine. Among the Basidiomycota, five species of the genus *Wallemia* were the most abundant, isolated from the majority of the samples studied.

Amplicon sequencing of the ITS2 region and sequence analysis by QIIME2 allowed us to detect 53 different fungal genera, including a large number of taxa that had not been previously isolated from the Sečovlje salterns. As expected due to the lower salinity, the largest number of different fungal genera were detected in seawater. Using QIIME2, alpha diversity indexes were calculated (Chao1, Shannon, Simpson, Faith-pd) but, according to the Kruskal-Wallis analysis of variance, differences were not statistically significant (p -value > 0.05). The values of the diversity index (Shannon) were lowest in the samples with moderate salinity (10 - 20 °Bé).

Session 9 - Biotechnology and Bioremediation

The cultivable mycobiota associated with the seagrass *Cymodocea nodosa* for ecological and biotechnological purposes

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Oceans are characterized by high pressure, low temperatures, low pH, high salinity, darkness and permanent exposure to ocean dynamics, which translate into hostile environments, compared to most terrestrial habitats, for the evolution of life. A broad knowledge is available on the diversity and abundance of terrestrial fungi, as well as on their implications and potential in crucial aspects as human and animal health, biotechnology and environmental management, among others. In contrast, their marine counterparts are scarcely studied. This raises certain doubts regarding fungal communities in these almost extreme environments such as halophilic habitats: Are we underestimating the role of fungal communities in marine ecosystems and their potential as a bioresource?

Marine fungi are widespread in the oceans. Moreover, their association with the water column, sediments and organisms of all trophic levels has been described, where they can act as parasites, pathogens, saprophytes or symbionts, which evidences the wide range of possible implications and the potential of marine fungal diversity. However, the magnitude of their abundance and diversity is still unknown. This study aims to provide further knowledge that help to answer the question posed. In the present work, the cultivable epiphytic and endophytic mycobiota associated with the marine plant *Cymodocea nodosa* that forms key habitats in the coastal ecosystems of the eastern Atlantic have been assessed. We identified more than 100 taxa (mainly Ascomycota), by the mean of a polyphasic approach, including the strictly marine fungus *Cumulospora marina*. *Cumulospora marina* has been determined as an endophyte associated with *Cymodocea nodosa*. The results obtained establish a framework to provide more information on the implications of the strictly marine fungi isolated in the promotion of the growth of this seagrass, as well as the discovery of possible new biomolecules for biotechnological purposes.

Degradation of polypropylene by fungi from hydrocarbon-contaminated environments

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Since mass production of plastics began in the 1950s, more than 9 billion tons of plastic have been produced, with about 75% of plastic waste ending up in landfills or nature. The accumulation of plastics in the environment has also significant impacts on human and animal health. Therefore, there is an urgent need to find new ways to manage plastic waste. One important but little-researched method is the biodegradation of plastics by microorganisms, which is also one of the more environmentally friendly alternatives to current methods. It is known that many microorganisms can metabolize different types of plastics. However, little is known about the biodegradation of polypropylene, one of the most commonly used plastics. Here we report the isolation and characterization of two fungal strains, namely *Coniochaeta hoffmannii* and *Pleurostoma richardsiae*, that have the potential to degrade pure polypropylene. First, twenty-seven fungal strains, many of which were isolated from sites contaminated with hydrocarbons, were initially screened for degradation of commercial textile polypropylene. Two of the strains, identified as *C. hoffmannii* and *P. richardsiae*, colonized the polypropylene fibers, as determined by scanning electron microscopy (SEM). Further experiments to study the degradation of pure polypropylene films were performed using Raman spectroscopy and Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR). The results showed that the selected fungi were active against pure polypropylene, with marked differences in the bonds attacked and the extent to which they were altered. Enzymatic tests were performed to evaluate the ability of each strain to degrade both naturally occurring compounds and synthetic polymers. These tests revealed possible adaptations to hydrocarbon-rich environments and provide a basis for further investigation of the polypropylene-degrading activity of *C. hoffmannii* and *P. richardsiae*.

Use of the industrial surfaces biofilm reactor to grow *Aureobasidium pullulans*-dominated biofilms: understanding biodeterioration of building materials

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Microbial colonization on building materials is a common issue that may result in the impairment of the aesthetic quality of buildings, deterioration and accelerated weathering of materials affecting their durability, and potential health risks for humans (*e.g.*, some microbes might be allergenic). Fungi, particularly polyextremophile black yeast-like fungi such as *Aureobasidium pullulans*, have been commonly found within biofilms formed on outdoor building materials. In this study we investigated the surface colonization and biofilm development of common microorganisms found on building materials, including fungi, algae and cyanobacteria on wood samples. Material biodeterioration — also known as defacement — was evaluated by accelerated tests in a novel bioreactor system, the industrial surfaces biofilm reactor (ISBR), designed to be operated as a high humidity/partially-wetted system to ensure microbial germination and growth. Wood samples were inoculated with spores of *A. pullulans* and autotrophic microorganisms (*Chlorella vulgaris*, a microalga; *Anabaena* sp. a cyanobacterium isolated from soil) and incubated at room temperature ($23\pm 1^\circ\text{C}$) and relative humidity $>95\%$; periodic wetting of the samples with growth medium was performed via a misting system. Microbial proliferation and material defacement were analyzed weekly for four weeks. Digital image processing was used as a tool for assessment and analysis of material defacement. Fungi and autotrophic microorganisms were successfully established on the wood panels, with *A. pullulans* as the dominant species. The ISBR successfully mimicked biofilm development on building materials; this system could be used to test the biodeterioration of building materials under a broad range of operational conditions (relative humidity, temperature, simulation of rain events, light intensities, etc.) that might influence microbial proliferation and biofilm development.

***Aureobasidium pullulans* versus *Aureobasidium melanogenum* - the impact of climate change on the fungal biodiversity variation**

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Aureobasidium spp. are widely distributed and versatile yeast-like fungi that frequently colonize various surfaces. *A. melanogenum* and *A. pullulans* are prevailing species within this genus, commonly encountered on wood surfaces. The highly successful colonisation is related to their remarkable abilities to adapt to different climatic conditions and low nutrient requirements. The climate of Earth has exhibited a pattern of historical variations, featuring periods of cold global temperatures interspersed with periods of warming. Knowledge on the impact of climate change on fungal diversity and adaptation is fundamental for comprehending the colonization dynamics. This study aims to explore the response of *A. pullulans* and *A. melanogenum* to climate change. Understanding colonization dynamics and interactions with wood materials will provide valuable insights for the development of targeted and effective wood protection strategies.

A. melanogenum was found the most commonly occurring dominant species on outdoor cladding materials based on the study of 34 various biobased samples vertically exposed in four cardinal directions. A noticeable increase of the number of samples where *A. melanogenum* was identified as the dominant species was observed within 4 months of exposure. The overall fungal burden has likewise increased over time. The differences in colonization rate were only observed after the first month, appearing less evident with the progress of weathering. *A. melanogenum* can tolerate higher temperatures and produces more melanin, making it more resistant to UV radiation which could be among the reasons for its prevalence on cladding materials. Since the temperature is rising and extreme weather is becoming more frequent, *A. melanogenum* has a good chance to continue leading this race. While is considered an opportunistic pathogen, this is something we may wish to monitor more closely in the future.

Valorization of agro-industrial byproducts by novel *Rhodotorula* yeasts for the production of value-added products.

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Rhodotorula yeasts are a novel yeast genus with the potential of being a powerhouse microorganism. These carotogenic yeasts can serve as highly efficient microbial factories for the production of a wide range of biomolecules, with applications in the food and feed industry and the pharmaceutical industry, as well as a source of biofuels. Within the framework of circular bioeconomy, low-cost agroindustrial byproducts hold significant promise as an alternative substrate for bioprocesses. The objective of this study was to assess the potential of Brewer's Spent Grain (BSG), the major by-product of the brewing industry, as a growth medium for four *Rhodotorula* strains provided by the Microbial Culture Collection Ex of the of Infrastructural Mycosmo Centre. *R. diobovata* EXF-6843, *R. kratochvilovae* EXF-3471, *R. mucilaginosa* EXF-8984 and *R. mucilaginosa* EXF-9791 were examined as single cell oil (SCO) producers cultivated on rich in free sugars BSG aqueous extract (RS~50-60 g/L) and glucose (as control culture). Among the yeast tested, *R. kratochvilovae* EXF-3471 performed as the best intracellular lipid producer both when cultivated in BSG and glucose (20.6% DCW and 43% DCW, respectively) and *R. diobovata* EXF-6843 presented the highest DCW, 9.8 g L⁻¹ on BSG and 12.7 g L⁻¹ on glucose. An unexpected discovery was the unmistakable aroma of citrus leaves when the yeasts were cultivated in BSG, especially in the *R. mucilaginosa* strains cultures. This study demonstrated the effective utilization of byproducts from beer-brewing as valuable renewable resources for the production of value-added products by novel *Rhodotorula* yeasts.

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***Exophiala* CAZymes genome annotation shows enzymes linked to opportunistic abilities**

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The genus *Exophiala* (Herpotrichiellaceae) comprises opportunistic species, causing chronic infections in humans and animals. as well as strict saprobes with niches related to extreme environments, like those polluted with aromatic compounds. CAZymes are involved in the fungal ability to colonize different habitats, including animal hosts. Therefore, understanding of CAZyme variety in members of *Exophiala* from different niches can help to elucidate pathogenic routes. Genome annotation of 11 *Exophiala* species available in NCBI (7 related to opportunistic infection and 4 strictly saprobic) was performed with Funannotate pipeline v1.7.0. CAZymes annotation was assigned according to Pfam database and analyzed manually. A total of 116 enzymes were annotated, of which 105 are found in both saprobes and opportunists, while 4 enzymes are found only in saprobes (GH11, GH109, GH135, GH141), and 7 enzymes are found only in opportunists (GT5, GH49, GH88, GH92, GH140, GH145, GH146). The opportunistic strains show enzymes that have important catalytic activity in carbohydrates presented in vertebrates. For example, GH92 (in *Exophiala oligosperma*) with catalytic activity on α -1,2-, α -1,3-, α -1,4- and α -1,6-linked mannose producing D-mannopyranose, which plays a role in glycosylation of proteins, and GH49 (presented in *Exophiala xenobiotica*) that has cleavage activity of α -1,6-glucosides and consequently, catalytic activity in glycogen, which stored energy in the liver and the muscle in vertebrate animals. Therefore, although *Exophiala* CAZymes profiles are relatively conserved among strains presenting similar niches regarding carbohydrate metabolism, some species contained enzymes with catalytic activity in glycoproteins and polysaccharides presented in vertebrates, which could explain their opportunistic ability.

World Unique Culture Collection of Extremophilic Microorganisms Ex

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The microbiological collection Ex is an important part of the Mycosmo Infrastructural Centre (IC Mycosmo), which is operated within the Network of Research and Infrastructural Centres of the University of Ljubljana. The Ex-culture collection, established in 1998, is located at the Department of Biology of the Biotechnical Faculty of the University of Ljubljana. It currently includes 17,500 fungal, 6,300 bacterial, 50 archaeal and 9 algal strains, the majority is in the public domain. Data on the cultures are published in the online catalogue on the website (<http://www.ex-genebank.com/>). To manage all data on the strains, we use Biotree software, specially adapted for our collection.

The Ex collection is unique in the world in that it specialises in extremophilic fungi isolated from various extreme environments. These include extremely cold environments such as polar and high mountain glaciers and ice, snow, seawater, and extremely saline environments such as salt pans and salty lakes. Strains of extremophilic fungi have also been isolated from hot and cold deserts, geysers, the deep sea, and extremely nutrient-poor environments such as rocks and water. In recent years, we have enriched the collection with strains isolated from extreme domestic environments, especially from household appliances and kitchens. We have also collected many fungal strains from Slovenian cultural heritage objects and sites. Our extensive sampling resulted in a unique collection of bacteria and fungi from Slovenian natural heritage, the endemic troglomorphic aquatic salamander *Proteus anguinus*.

The main task of the collection, in addition to the isolation of microorganisms, is the identification and genetically stable storage of microorganisms. Cultures are stored mainly in the freezer (-80°C) and in liquid nitrogen (-140 to -196°C), and some are kept refrigerated on slanted culture media. Most cultures in the Ex collection are identified by DNA sequence barcodes, more than 200 strains are also genome sequenced.

Fungus from a historical copper-impregnated timber bridge in northern Sweden

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The wooden bridge Leonstrom was built in Skelleftea, Northern Sweden, in 1737. The bridge underwent construction changes in 1868 to enhance its structural integrity. The changes included the use of a truss structure and the replacement of wooden boxes with stone pillars to withstand heavy stresses better. However, significant decay was discovered during an annual inspection in 2017. Large-scale computer tomography revealed regions of lower wood density, mainly near the heads of the nails where water had penetrated.

To determine the cause of the density changes, the decay was found. To confirm the hypothesis, the wood material in these areas was surface sterilized and the fungal isolate was found to be grown on nutrient agar. The fungus aggressiveness was assessed for the decay intensity of copper-impregnated wood that was used for the replacement of degraded parts. The decay test was run according to EN113 standard. Small wooden pieces were placed on the grown fungal colony on nutrient media and further incubated for 16 weeks to evaluate the mass loss. Spectroscopic analysis indicated the absence of acetyl groups after decay, while mechanical tests showed a reduction in load and modulus of elasticity in originally degraded beams.

Biodegradation of ^{14}C -phenanthrene by the newly isolated marine-derived fungus, *Aspergillus welwitschiae* strain bpo1 using response surface methodology

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Removal of polycyclic aromatic hydrocarbon is of immense interest to researchers due to their teratogenic and mutagenic properties. This study investigated the removal of ^{14}C -phenanthrene degradation efficiency of marine-derived filamentous fungus, *Aspergillus welwitschiae*. Response Surface Methodology (RSM) using Box–Behnken Design (BBD) was successfully deployed in the optimization of process parameters (pH-6, temperature-30°C, substrate concentration-50 mg L⁻¹ and dry weight-1.5 g) resulting in 97.40% phenanthrene degradation on 6th day. The design and regression model were found to be statistically significant, adequate and appropriate with $p < 0.0001$, F value = 304.19, and predicted coefficient of determination ($R^2=0.9561$). Optimization of the vital constituents of the mineral salt medium (MSM) used for the study using RSM-Central Composite Design (CCD) resulted in 98.80% phenanthrene degradation rate. Enhanced phenanthrene degradation efficiency (99.50%) was recorded when the optimized process variables were subjected to growth-linked validation experiments. The enzyme assay experiments revealed 91%, 78% and 42% induction of laccase, manganese peroxidase and lignin peroxidase respectively. Metabolites obtained after the experiment were characterized and confirmed with GC-MS analysis. The findings revealed the promising potentials of fungi in green remediation technology.

Unravelling the xenome of *Debaryomyces hansenii* during benzo(a)pyrene degradation

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Polycyclic aromatic hydrocarbons (PAHs), like benzopyrene (BaP), are considered as pollutants that contaminate soils and aquatic niches, they are found ubiquitously and are considered extremely toxic. The need for new strategies using microorganism for remediation has led us to search for a yeast capable of growing in the presence of BaP. The marine and extremophilic yeast *Debaryomyces hansenii* has been show capability to grow in the presence of BaP at temperatures below its optimal. Preliminary results showed that BaP degradation depends on the concentration and amount of yeast, which through an oxidation process mediated by a cytochrome P450 (CYP), encoded by the *DhDIT2* gene, carries out BaP metabolism. The relative expression of genes involved in BaP metabolism during its degradation process was also studied. These results allow us to decipher the xenome of *D. hansenii* to obtain information on the BaP oxidation process, and thus accelerate the practical application of organisms such as this extremophilic yeast in the mycoremediation process.

Degradation of polypropylene by fungi *Coniochaeta hoffmannii* and *Pleurostoma richardsiae*

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The urgent need for better disposal and recycling of plastics has motivated a search for microbes with the ability to degrade synthetic polymers. While microbes capable of metabolizing polyurethane and polyethylene terephthalate have been discovered and even leveraged in enzymatic recycling approaches, microbial degradation of additive-free polypropylene remains elusive. Here we report the isolation and characterization of two fungal strains with the potential to degrade pure polypropylene. Twenty-seven fungal strains, many isolated from hydrocarbon contaminated sites, were screened for degradation of commercially used textile plastic. Of the candidate strains, two identified as *Coniochaeta hoffmannii* and *Pleurostoma richardsiae* were found to colonize the plastic fibers using scanning electron microscopy (SEM). Further experiments probing degradation of pure polypropylene (PP) films were performed using *C. hoffmannii* and *P. richardsiae* and analyzed using SEM, Raman spectroscopy and Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR). The results showed that the selected fungi were active against pure polypropylene, with distinct differences in the bonds targeted and the degree to which each was altered. Whole genome and transcriptome sequencing was conducted for both strains and the abundance of carbohydrate active enzymes, GC content, and codon usage bias were analyzed in predicted proteomes for each. Enzymatic assays were conducted to assess each strain's ability to degrade naturally occurring compounds as well as synthetic polymers. These investigations revealed potential adaptations to hydrocarbon-rich environments and provide a foundation for further investigation of polypropylene degrading activity in *C. hoffmannii* and *P. richardsiae*.

Screening of fungi isolated from sewage sludge and composting processes to assess their potential as plastic and related compounds degraders

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Prediction about plastic waste suggest that in 2050 the plastic waste production in ecosystems will reach over 25 billion tons. The degradability of these polymers depends on crystallinity, shape, size, chloride atoms, benzene rings or molecular weight. One of the strategies in the search of microorganisms with abilities to degrade these compounds is focused directly on the isolation of strains from environment polluted with plastics. Fungi may be a feasible candidate for the plastic degradation due to their extracellular oxidation mechanisms through the secretion of extracellular and intracellular enzymes. In this study, the main objective is the search of fungal strains with abilities to degrade plastics or plasticizers. To reach this objective, we have evaluated the potential of fungal strains from a collection obtained from a sewage sludge composting pile and from a digested sewage sludge. Two different methods were considered using 1) Petri plate with different plastic compounds and a minimal medium for plastics and 2) the method of the microplate for the plasticizer-derivate compounds. After 60 days of incubation the pieces of plastics were washed with distilled water to remove the remaining mycelium, weight out and analyzed by Atomic Force Microscopy (AFM). Plasticizer like bisphenol A (BPA) and Bis(2-ethylhexyl) phthalate (DEHP) were analyzed by liquid chromatography (HPLC). The results of Petri dishes showed growth using the xenobiotic as solely carbon source for *Aspergillus tubingensis* and *Thielavia* sp. The results of AFM are being currently analyzed to elucidate the affected area of the plastics. The non-identified fungi from digested sewage sludge showed a growth on the microplate suggesting the use of the carbon provided from BPA and DEHP. The determination of the concentration of these compounds is needed to determine the potential of these fungi to degrade both compounds.

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The extreme environment is closer than you think – the ARCHI-SKIN concept for the protection of building facades

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The global average temperature is expected to reach or exceed 1.5°C within the next few decades, which will affect all regions of the World. Earth is already facing extreme global weather conditions, such as heavy precipitation and heat waves, which will recently become more apparent, frequent, widespread, and intense. By 2050 the global population will rise to 9.7 billion people who will need a place to live. Over half of the world's inhabitants live in cities occupying buildings exposed to environmental and biotic degradation. Architectonic coatings enhance the functional and aesthetical durability of building materials by protecting their surface. Consequently, paints and coating applied on the exterior of a residence, or a commercial building not only add to the aesthetics of the structure but also defend it against heat, UV, harsh winters, soaking rain, and other adverse weather conditions. The commonly used surface treatments consist of mineral oil binders and many environmentally unwanted ingredients, therefore environmentally friendly alternatives are within the interest of architects, constructors and end-users.

The ARCHI-SKIN project takes a bio-inspired approach and aims to generate completely new solutions for architectural coatings. A prototype microbial coating based on biofilm built by the ubiquitous, yeast-like, oligotroph fungus, *Aureobasidium pullulans* is under development. The concept is based on a technically applicable, controlled, and optimized biofilm formation that effectively protects the substrate surfaces, assuring optimal service life performance and different functionalities including self-healing. This contribution presents ambitions, challenges, and first results related to the development of a living coating system for architectonic surfaces.

Taxonomic diversity of the genus *Aureobasidium* exploited for biotechnology

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The genus *Aureobasidium* represents a group of highly versatile yeast-like fungi. They show great adaptability to different environments and can be found in diverse ecological niches, from cold or temperate regions, across humid and tropical areas, to warm and dry habitats. Since *Aureobasidium* is able to tolerate different stresses like hypersalinity, extreme temperatures, and oligotrophic conditions, the extremophilic fungus provides great biotechnological potential.

To explore the whole genus in more depth and to identify strains for potential biotechnological applications, we performed an extensive screening with 128 strains from nine *Aureobasidium* species. We collected 89 strains from two strain collections and locally isolated 39 new wild-type strains from different natural and anthropogenic environments. Three highly conserved genome regions were sequenced for each strain and a phylogenetic tree was constructed based on the results. The tolerances of the strains towards extreme pH values, high salt concentrations, high and low temperatures, and UV radiation were tested and added to the phylogenetic tree to identify differences within the genus and find strains for potential industrial applications.

In addition, we analyzed the different morphologies of the strains and tested different carbon sources important in the context of the circular bioeconomy to emphasize the suitability for biotechnology. The majority of strains were able to grow on a variety of lignocellulose compounds, the most abundant raw material on earth. Furthermore, most strains could grow on acetate as a carbon source, which is a sustainable carbon source since it can potentially be produced from CO₂.

Overall, we created an extensive overview of the genus *Aureobasidium* and identified strains with interesting biotechnological traits that offer the potential for more detailed investigations. With the help of the phylogenetic tree, we want to gain more insights into the specific genetic background of selected traits.

Assessing the role of Hog1 in lipid accumulation in the osmotolerant yeast *Debaryomyces hansenii*

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Lipid synthesis by yeast has gained attention as a sustainable alternative for producing oleochemicals. Nonetheless, the mechanisms involved in regulating the lipid metabolism of oleaginous yeasts (OY) in stressful conditions still need to be better understood. In the non-oleaginous yeast *Saccharomyces cerevisiae*, the MAP kinase Hog1 is required for neutral lipid (NL) accumulation, while in the opportunistic pathogen yeast *Candida albicans*, Hog1 down-regulates lipid synthesis. However, it is unknown whether Hog1 is involved in the *de novo* lipid synthesis of *Debaryomyces hansenii*, an osmotolerant yeast that stores NL under nitrogen-limited conditions. This study aims to characterize the synthesis of NL in response to nitrogen limitation and determine if *DhHog1* contributes to lipid accumulation in *D. hansenii* by comparing a wild-type strain with a *Dhhog1Δ* mutant. We determine the fatty acid (FA) profile and quantify NL by GC-MS and flow cytometry, respectively. Our results suggest that the most significant accumulation of NL occurs during the stationary phase, in which unsaturated FAs represent the bulk of the lipids produced under nitrogen-limited conditions. The expression of several genes involved in the *de novo* lipid synthesis pathway is also analyzed by RT-qPCR. Additionally, we look up whether the ATP-citrate lyase enzyme (Acl) is present or absent in *D. hansenii*, as Acl provides the necessary acetyl-CoA for lipid synthesis in many OY under lipogenic conditions. We will discuss the role of *DhHog1* in the lipid accumulation of *D. hansenii*, which could help understand the relationship between stress and lipid metabolism.

Mycoremediation potential of *Cephalotrichum* sp. MUT6686 (Sordariomycetes; Microascaceae) isolated from a site heavily contaminated by petroleum hydrocarbons

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The transformation of polycyclic aromatic hydrocarbons (PAHs) by microfungi that tolerate extreme environmental conditions may be of outstanding importance for mycoremediation of PAH-polluted sites. *Cephalotrichum* sp. MUT6686 (Microascaceae) was isolated from a refined oil stocking areas in Italy. *Cephalotrichum* sp. MUT6686 becomes black for the melanin production ability, which defends against extreme environmental stresses, and can grow in presence of different hydrocarbons as a sole carbon source. Moreover, *Cephalotrichum* sp. MUT6686 did not exhibit resistance to different antifungal agents (ciclopirox, fluconazole and tioconazole), but very little information exists on the mycotoxin production.

Modern DNA sequencing technologies can help to study the potential of extremophilic and extreme-tolerant fungi that survive in environments with high concentration of pollutants. In this study, the genome of *Cephalotrichum* sp. MUT6686 (Bioproject PRJNA850907; BioSample SAMN29205113) was sequenced, assembled and annotated (35.9 Mb, GC% 55.26, 10,597 genes). We investigated the occurrence of genes associated to PAH tolerance and potentially involvement in metabolism of PAHs. Moreover, the genome of *Cephalotrichum* sp. MUT6686 was compared with *Scedosporium* genomes (Microascaceae) previously sequenced for a better investigation of their high degrading potential in PAH-polluted environment and industrial wastewater.

In silico analysis of the MUT6686 genome predicted genes potentially involved in the biodegradation pathway for PAHs. The genes encoding enzymes for the enhanced solubility of PAHs, which could be included in the PAH degradation pathway, were found in the MUT6686 genome. Secondary metabolism (SM) biosynthetic gene clusters (BGCs), which may be of relevance to mycoremediation, were also investigated. The identification of genes encoding hydrocarbon degrading enzymes and a low diversity in SM BGCs indicate the potential value of MUT6686 for mycoremediation. However, it could not be considered for mycoremediation of sites contaminated by azole and synthetic antifungal drugs. Future work will be aimed to ascertain the PAH biodegradation capability of MUT6686 in PAH-contaminated soil microcosms via transcriptomics approaches.

Potential new *Aspergillus* species inhabit halites at Salar Grande, Atacama Desert

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High desiccation and solar irradiation are the two most important stressors to life in the Atacama Desert. In Salar Grande, a large coastal saltflat, halite rocks are colonized with halophilic and halotolerant microbial consortia adapted to grow inside and within the halite rocks. Culture-dependent evidence on fungi biodiversity are scarce in such extreme habitat. Then, we have collected halites to grow and isolate fungi from the lithobiontic community. Samples were grown in extract malt broth at 28°C and 120 rpm during 7-14 days. Morphological fungi characterization was performed in isolates cultured in agar plates in three culture media (potato dextrose, oatmeal, and Dichloran Glycerol agar). Molecular analyses were conducted by amplification and sequencing of *BenA*, *CaM* and ITS loci. From a total of 22 fungal isolates obtained, eleven belonged to the genus *Aspergillus* which were distributed into three sections and series of the genus, and classified into seven different species. Isolates inhabiting Salar Grande halites were distributed into sections *Aspergillus*, *Nidulantes* and *Nigri*, and series for *Aspergillus*. These fungal isolates colonize a habitat under multi-extreme environmental conditions, may represent new *Aspergillus* species with biotechnological potential; then, a full characterization is required, which includes sequencing the *rpb2* marker, chemical, and morphology studies.

Production of pathogenic airborne fungal spores under pollution stress

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Organic pollutants can enter all environmental niches. We are willing to better understand the interplay between pollution and fungal virulence. In previous work we demonstrated that a metacommunity of fungi was able to efficiently degrade the persistent organic pollutant pentachlorophenol. The cost is high though, since degradation elicited pathogenic trade-offs, namely the dysregulation of the C and N metabolisms, the secretion of proteins associated with pathogenesis and a substantial decrease in the susceptibility of the metacommunity to a common fungicide. In a subsequent study, we demonstrated that short-term (acute) exposure to aromatic hydrocarbon pollutants - pentachlorophenol and triclosan - increased the virulence of the produced airborne fungal spores. Each pollutant altered the composition of the community of airborne spores, favouring an increase in strains with higher *in vivo* infection capacity (*Galleria mellonella* as infection model). Moreover, fungi subsisting inside larvae infected with the airborne spores produced in a polluted environment, 72h post-infection, comprised several virulent *Aspergillus* strains. On the contrary, only no virulent strains could be isolated from the larvae infected with airborne spores from an unpolluted condition. None of the observed taxonomic or functional traits investigated could separate the virulent from the avirulent fungal strains. Laboratory evolution of selected fungal strains (polluted vs unpolluted conditions) were designed to mechanistically understand why pollution-stress is acting as a driver of phenotypic adaptations that increase *Aspergillus* spp. pathogenicity.

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Legend: keynote= key note lecture; keynote-end= key note lecture at the end of the conference; IL=invited lecturer; S=speaker; P=poster; presentations, presented by the listed author are **in bold**