Programme

1st Mycology Tyrol Mini-Symposium

29th September 2017
Welcome

The 1st Mycology Tyrol Mini-Symposium will create a highly interdisciplinary scientific exchange and discussion platform on topics including Molecular Fungal Cell Biology, Fungal Physiology, Environmental Mycology, Microbial Resource Management and Clinical Mycology. Researchers from the University of Innsbruck and the Medical University of Innsbruck will join forces to present their latest advancements in these fields and discuss novel trends. In addition to plenary sessions, Ph.D. students and postdoctoral researchers will have the opportunity to present their projects in short elevator talks and receive feedback from the community in open poster sessions. The concluding open mixer will provide time for extensive networking and for finding collaboration opportunities. This one-day symposium is free and open to everyone with a special interest in modern mycological research.

We wish all attendees a productive and enjoyable day.

For additional information please visit www.mycologytyrol.org.

hosted by the
Institute of Microbiology
University of Innsbruck
Technikerstrasse 25, A-6020 Innsbruck
wwwuibk.ac.at/microbiology/index.html.en
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Chair</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00 – 9:15</td>
<td>Opening Remarks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:15 – 10:15</td>
<td>Plenary Session 1</td>
<td>chaired by Ursula Peintner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:15</td>
<td>Ulrike Binder</td>
<td>Division of Hygiene and Medical Microbiology, MUI</td>
<td>Galleria mellonella: an alternative infection model</td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td>Hermann Strasser</td>
<td>Institute of Microbiology, LFU</td>
<td>Innovation in Biocontrol – Challenges and Research: Making fungi - as biocontrol option - more accessible to farmers</td>
<td></td>
</tr>
<tr>
<td>9:45</td>
<td>Florentine Marx</td>
<td>Division of Molecular Biology, MUI</td>
<td>Small, cysteine-rich, cationic proteins from Penicillium chrysogenum: antimicrobial potential and mode of action</td>
<td></td>
</tr>
<tr>
<td>10:00</td>
<td>Wolfgang Burgstaller</td>
<td>Institute of Microbiology, LFU</td>
<td>Excretion of organic acids by <em>Penicillium ochrochloron</em></td>
<td></td>
</tr>
<tr>
<td>10:15 – 10:35</td>
<td>Poster Session 1</td>
<td>chaired by Hoda Bazafkan &amp; Stefan Ciaghi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anna Huber</td>
<td>Division of Molecular Biology, MUI</td>
<td>The antimicrobial mode of action of PAFB, a second small, cysteine-rich and cationic protein from <em>Penicillium chrysogenum</em> Q176</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Christoph Sonderegger</td>
<td>Division of Molecular Biology, MUI</td>
<td>Design and characterisation of improved antifungal peptides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maria Zottele</td>
<td>Institute of Microbiology, LFU</td>
<td>Advances in the development of novel environmentally friendly control agents based on entomopathogenic fungi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Georg Walch</td>
<td>Institute of Microbiology, LFU</td>
<td><em>Stachybotrys chartarum</em> and its mycotoxins: A risk assessment of living in moldy homes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maria Salgado</td>
<td>Forestal Center CIEFAP. National University of Patagonia</td>
<td><em>Cortinarius magellanicus</em> Spegazzini and its look-alikes: the most widely distributed edible <em>Cortinarius</em> of Patagonia is a species complex.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daniela Kasakova</td>
<td>Slovak University of Technology</td>
<td>Effect of deletion <em>gad1</em> and <em>gad2</em> genes on growth and development of <em>Neurospora crassa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:35 – 11:00</td>
<td>Coffee Break with Poster Viewing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td>Plenary Session 2</td>
<td>chaired by Sigrid Neuhauser</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td>Martin Kirchmair</td>
<td>Institute of Microbiology, LFU</td>
<td>Indoor mycology</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Speaker</td>
<td>Institution</td>
<td>Title</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------</td>
<td>----------------------------------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>11:15</td>
<td>Michaela Lackner</td>
<td>Division of Hygiene and Medical Microbiology, MUI</td>
<td>Understanding antifungal resistance and improving fungal diagnostics</td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td>Susanne Zeilinger</td>
<td>Institute of Microbiology, LFU</td>
<td>Molecular mechanisms of the mycoparasitic fungus - fungus interaction</td>
<td></td>
</tr>
<tr>
<td>11:45</td>
<td>Andreas Wagner</td>
<td>Institute of Microbiology, LFU</td>
<td><em>Trichoderma viride</em> biowaste pre-treatment for enhanced biogas production</td>
<td></td>
</tr>
</tbody>
</table>

**12:00 – 12:20 Poster Session 2** chaired by Julia Badstöber & Philipp Dresch

<table>
<thead>
<tr>
<th>Speaker</th>
<th>Institution</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rudi Markt</td>
<td>Institute of Microbiology, LFU</td>
<td><em>Trichoderma viride</em> bio-waste pre-treatment for enhanced biogas production</td>
</tr>
<tr>
<td>Antonio Perez Hansen</td>
<td>Division of Hygiene and Medical Microbiology, MUI</td>
<td>Resistance screening of commonly used antimycotics in rare yeasts</td>
</tr>
<tr>
<td>Dubraska Moreno-Ruiz</td>
<td>Institute of Microbiology, LFU</td>
<td>Chemotrophic growth of <em>Trichoderma atroviride</em> towards host-derived compounds is not affected by deletion of the <em>tmk1</em> MAPK gene</td>
</tr>
<tr>
<td>Alex Lichius</td>
<td>Institute of Microbiology, LFU</td>
<td>An improved CRIB reporter enables the identification of individual hyphae of <em>Trichoderma atroviride</em> engaged in mycoparasitic attack</td>
</tr>
<tr>
<td>Verena Speckbacher</td>
<td>Institute of Microbiology, LFU</td>
<td>Is a lipoxygenase involved in 6-Pentyl-α-Pyrone biosynthesis in <em>Trichoderma atroviride</em>?</td>
</tr>
<tr>
<td>Hoda Bazafkan</td>
<td>Institute of Microbiology, LFU</td>
<td>TOR kinase signaling in the mycoparasite <em>Trichoderma atroviride</em></td>
</tr>
</tbody>
</table>

**12:20 – 13:00 Lunch Break**

**13:00 – 14:00 Plenary Session 3** chaired by Michaela Lackner

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:00</td>
<td>Pamela Vrabl</td>
<td>Institute of Microbiology, LFU</td>
<td>Triggering bioactive metabolite production in fungi</td>
</tr>
<tr>
<td>13:15</td>
<td>Bianka Siewert</td>
<td>Institute of Pharmacy - Pharmacognosy</td>
<td>Colored Fungal Metabolites: A new Source of Photoactivatable Drugs?</td>
</tr>
<tr>
<td>13:30</td>
<td>Hubertus Haas</td>
<td>Division of Molecular Biology, MUI</td>
<td>Iron homeostasis - a key hub in virulence of <em>Aspergillus fumigatus</em></td>
</tr>
<tr>
<td>13:45</td>
<td>Ursula Peintner</td>
<td>Institute of Microbiology, LFU</td>
<td>Mycological Systematics – dead or alive? What about biodiversity, function and interactions without knowing fungi?</td>
</tr>
</tbody>
</table>
### 14:00 – 14:20  Poster Session 3  chaired by Verena Speckbacher & Alex Lichius

<table>
<thead>
<tr>
<th>Name</th>
<th>Institute</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desiree Artmann</td>
<td>Institute of Microbiology, LFU</td>
<td>Searching for (photosensitive) bioactive fungal metabolites via a modified antimicrobial EUCAST susceptibility test</td>
</tr>
<tr>
<td>Philipp Dresch</td>
<td>Institute of Microbiology, LFU</td>
<td>Primary Succession of Active Fungal Communities in a Glacier Foreland</td>
</tr>
<tr>
<td>Thomas Orasch</td>
<td>Division of Molecular Biology, MUI</td>
<td>The <em>Aspergillus fumigatus</em> leucine biosynthesis-regulator LeuB is crucial for adaptation to iron starvation and virulence in <em>Galleria mellonella</em></td>
</tr>
<tr>
<td>Anna-Maria Dietl</td>
<td>Division of Molecular Biology, MUI</td>
<td>Identification and characterization of antifungal drug targets in <em>Aspergillus fumigatus</em></td>
</tr>
<tr>
<td>Manuel Sanchez</td>
<td>Division of Molecular Biology, MUI</td>
<td>The bZIP transcription factor HapX is regulated at multiple levels to control iron homeostasis in <em>Aspergillus fumigatus</em></td>
</tr>
<tr>
<td>Ulrike Binder</td>
<td>Division of Hygiene and Medical Microbiology, MUI</td>
<td>Bioluminescent <em>Mucor circinelloides</em> – a promising new tool to study mucormycosis and antifungal drug efficacy</td>
</tr>
</tbody>
</table>

### 14:20 – 14:45  Coffee Break with Poster Viewing

### 14:45 – 15:45  Plenary Session 4  chaired by Susanne Zeilinger

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:45</td>
<td>Sigrid Neuhauser</td>
<td>Phytomyxid-Host interactions – putting transcriptomic data under the microscope</td>
</tr>
<tr>
<td>15:00</td>
<td>Stefan Grässle</td>
<td>Histone deacetylases as drug targets and regulators of small bioactive molecules in filamentous fungi</td>
</tr>
<tr>
<td>15:15</td>
<td>Magdalena Nagler</td>
<td>Exploring the hidden biotechnological potential of anaerobic fungi (Neocallimastigomycota)</td>
</tr>
<tr>
<td></td>
<td>Cornelia Speth</td>
<td>Antifungal immune reaction of human platelets</td>
</tr>
</tbody>
</table>

### 15:45 – 16:05  Poster Session 4  chaired by Georg Walch & Dubraska Moreno-Ruiz

<table>
<thead>
<tr>
<th>Name</th>
<th>Institute</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingo Bauer</td>
<td>Division of Molecular Biology, MUI</td>
<td>AN4022 – A novel HDAC complex component as basis for antifungal therapy</td>
</tr>
<tr>
<td>Stefan Ciaghi</td>
<td>Institute of Microbiology, LFU</td>
<td>Transcriptomic interaction during clubroot disease development</td>
</tr>
<tr>
<td>Julia Badstöber</td>
<td>Institute of Microbiology, LFU</td>
<td>Studying phytomyxid-host interactions with <em>in-situ</em> monitoring of gene expression</td>
</tr>
<tr>
<td>Mohammad Etemadi</td>
<td>Institute of Microbiology, LFU</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Department</td>
<td>Topic</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Günter Rambach</td>
<td>Division of Hygiene and Medical Microbiology, MUI</td>
<td>Platelets as immune players in invasive <em>Candida</em> infections</td>
</tr>
<tr>
<td>Hemalata Deshmukh</td>
<td>Division of Hygiene and Medical Microbiology, MUI</td>
<td>Galactosaminogalactan secreted from <em>Aspergillus fumigatus</em> affects human platelet activity and stimulates complement system</td>
</tr>
</tbody>
</table>

from 16:05 Drinks & Networking
1.1

The antimicrobial mode of action of PAFB, a second small, cysteine-rich and cationic protein from *Penicillium chrysogenum* Q176

Anna Huber¹, Doris Bratschun-Khan¹, Mihayl Varbanov², Stephanie Philippot², László Galgóczy¹ and Florentine Marx¹

¹Division of Molecular Biology, Biocenter, Medical University of Innsbruck, Innsbruck, Austria  
²Faculté de Pharmacie, SRSMC, UMR 7565, Université de Lorraine-CNRS, Nancy Cedex, France.

Small, cationic and cysteine-rich antimicrobial proteins (AMPs) from filamentous ascomycetes represent promising bio-molecules for the development of novel antimicrobial drugs in medicine and agriculture. They are highly stable against harsh environmental conditions and some were identified to be non-toxic to mammalian cells *in vitro* and *in vivo* [1, 2].

The genome of the penicillin-producing mould *Penicillium chrysogenum* Q176 harbours more than one gene that code for secreted, small, cysteine-rich and cationic proteins. The structure and mode of action of one, the antifungal protein PAF, has been extensively examined [3, 4]. In this study, we analysed the antimicrobial activity of a second protein, PAFB, which is a prepro-protein with high similarity to the recently described protein PgAFP from *P. chrysogenum* R42C [5]. Although we detected timely regulated *pafB* gene transcripts in Northern blot experiments, we could not identify PAFB in the supernatant of *P. chrysogenum* Q176, cultivated under standard conditions. Therefore, we used our *P. chrysogenum*-based expression system to produce sufficient amounts of recombinant PAFB for functional characterization [6].

The purified, mature PAFB effectively inhibited the growth of human opportunistic pathogenic fungi like *Aspergillus fumigatus* and dermatophytes like *Trichophyton rubrum* in a µM concentration range. A potent killing activity was also identified against the yeast *Candida albicans*. Localization studies using fluorescence-labelled PAFB indicated that the fungicidal activity is closely linked with protein uptake and subsequent cytoplasmic localization in fungal cells. Finally, we report here for the first time the most interesting observation, that the *P. chrysogenum* AMPs have anti-viral activity.

1.2

Design and characterisation of improved antifungal peptides

Christoph Sonderegger1*, Györgyi Váradi2, László Galgóczy3, Florentine Marx1

1Division of Molecular Biology, Biocenter, Medical University of Innsbruck, Innsbruck, Austria
2Department of Medical Chemistry, Faculty of Medicine, University of Szeged, Szeged, Hungary
3Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

An exigent need for new antifungal therapeutics is corroborated by the fact that still 1.5 million people die each year due to fungal infections [1]. Current antifungals comprise only three drug classes: polyenes, azoles and echinocandins, which all show weakness in efficaciousness, therapeutic index and activity spectra [2]. Promising candidates for next generation therapeutics are peptide antifungals from natural source or rationally designed.

Based on the already comprehensively characterised antifungal protein PAF from *Penicillium chrysogenum* [4], we focused our study on the gamma(γ)-core protein motif, highly conserved in all classes of disulphide-stabilized antimicrobial peptides [3]. We synthesized peptides comprising the γ-core of PAF in its natural form (Py) and peptide variants with increased positive net charge and hydrophilicity (Pyvar & Pyopt). Interestingly, the short synthetic peptide Py alone exhibited antifungal activity on the PAF-sensitive yeast *Candida albicans*, with only a two-fold higher MIC (9 µM) than the whole protein (5 µM). This antifungal potential could be further increased with the peptide variants Pyvar and Pyopt, showing MIC’s of 2 & 1 µM respectively. The improved antifungal efficacy of Pyvar could be transferred to the whole protein by creating the PAF variant PAFyvar (MIC 1 µM). The killing mechanism of the proteins and peptides was closely linked to their active internalisation into *C. albicans* cells, the formation of reactive oxygen species and impairment of the cell membrane. Toxicity tests showed that none of the proteins or peptides was haemolytic or toxic to human cells in vitro.

Advances in the development of novel environmentally friendly control agents based on entomopathogenic fungi

Hermann Strasser, Christopher O. Pabst and Maria Zottele

BIPESCO Team Innsbruck, Institute of Microbiology, University Innsbruck, A - 6020 Innsbruck

This poster presents the experimental set up of two initiated projects: 1) MELOBEAUPOP: “Influence of spatial and temporal separation on population structure of the European cockchafer and its main fungal pathogen” (currently under review -Swiss National Fund) and 2) DIACONT: “Alternative methods for protection of maize from western corn rootworm (Diabrotica vigifera)”, funded by DaFNE No. 101111/2, since 2016.

MELOBEAUPOP: The overall objective is to resolve the influence of spatial and temporal isolation on the population structure of the May beetle Melolontha melolontha and its most important fungal antagonist Beauveria brongniartii in the Alpine region of Switzerland and Tyrol. It will be investigated, whether microbial communities, higher order phylogenetic groups or individual microbial populations, for instance native B. brongniartii populations affect establishment and development of applied B. brongniartii strains in soil and whether such strains are adapted to specific M. melolontha populations.

DIACONT: The main goal is to demonstrate the efficacy of three antagonistic organisms in controlling western corn rootworm (WCR) larvae or protecting corn roots. This should enable farmers to use at least three different biological methods and thereby decreasing dependency to only one active ingredient (i.e. Cypermethrin) in WCR control. BIPESCO team and partners aim to stimulate self-regulation of WCR by yearly prophylactic applications of small doses of our antagonistic organisms to the field. For Dianem® (Heterorhabditis bacteriophora, entomopathogenic nematode) which is already registered in Austria, the efficacy of practical applications by farmers will be correlated with agronomical and environmental factors in order to find out what promotes successful application. For GranMet® (Metarhizium brunneum, strain BIPESCO 5) field trials are ongoing to demonstrate the efficacy which has been shown in the laboratory very clearly. Concerning SPA69 (Stenotrophomonas rhizophila) a protecting agent for corn roots will be examined in field trials. The application of a combination of these organisms will also be performed in large field trails in 2018. In best case it will enable the registration of new plant protection products.

Notes


1.4

*Stachybotrys chartarum* and its mycotoxins: A risk assessment of living in moldy homes

Ralf Gebauer 1,2, Georg Walch 3, Sigrid Neuhauser 3, Johannes Rainer 4, Martin Kirchmair 3,4

1 both authors have contributed equally

1 Institute for Structural Engineering and Material Sciences, University of Innsbruck, A-6020 Innsbruck, Austria.
2 Sachverständige-Gebauer-Ingenieure, Schöffelhuberstraße 16, 82362 Weilheim, Germany.
3 Institute of Microbiology, University of Innsbruck, A-6020 Innsbruck, Austria.
4 Mykon _Technisches Büro für Baubiologie, Reimmichlstraße 9, A-6060 Hall, Austria.

The “black mold” *Stachybotrys chartarum* is found on wet gypsum boards, wallpapers and other materials containing high amounts of cellulose. Several strains of *S. chartarum* produce a wide variety of mycotoxins, among them macrocyclic trichothecenes that are potent inhibitors of protein synthesis. A potential relationship between *S. chartarum* growing on water-damaged surfaces in buildings and human illness caused by the inhalation of mycotoxins was discussed repeatedly. Based on literature and own data, we evaluated if *Stachybotrys* and its mycotoxins affect human health more than other indoor mold infestations. We calculated the number of conidia and airborne fungal fragments a person would have to inhale to reach the lowest effect level of trichothecenes (LOEL satratoxin G: 25 µg kg⁻¹ mouse, intranasal). Based on spore counts and toxin contents found on building materials, all of the fungal material formed on 2.8 cm² area of gypsum board has to be inhaled. According to literature, air in rooms with a heavy infestation contains 9.3 spores m⁻³. During remediation, spore counts can temporarily reach 8000 spores m⁻³. To be exposed to 25 µg of trichothecenes a person would have to inhale 5 000 000 m³ of air in a heavily infested but otherwise undisturbed room or 5900 m³ air during remediation. It would be necessary to spend decades or even centuries in such a room until the LOEL of *S. chartarum* toxins is inhaled. We therefore conclude that *Stachybotrys* spp. do not pose a risk more severe to human inhabitants than other indoor fungi. However, during remediation, high amounts of fungal propagules and other trichothecene-contaminated materials can become airborne. Consequently, the unprotected worker is at risk to inhale a critical amount of trichothecenes.

Notes
Cortinarius magellanicus Spegazzini and its look-alikes: the most widely distributed edible Cortinarius of Patagonia is a species complex.

María Eugenia Salgado Salomón1✉, Philipp Dresch2, Carolina Barroetaveña3, Úrsula Peintner2

1 Forestal Center CIEFAP. National University of Patagonia S.J. Bosco, Esquel, Chubut, Argentina.
2University of Innsbruck, Institute of Microbiology, Technikerstr. 25, 6020 Innsbruck, Austria
✉mesalgadosalomon@ciefap.org.ar

Cortinarius magellanicus Spegazzini (1887) is an edible mushroom, which is usually consumed in Patagonia. This beautiful mushroom can be recognized by the conspicuously violet, slender fruitbodies, which are glutinous all over, as typical for Cortinarius subgenus Myxacium. Spegazzini’s original material was lost; for that reason Cortinarius magellanicus was re-described and validated with a neotype by Moser & Horak (1975). Since then, Cortinarius magellanicus was considered a living fossil of Nothofagaceae forests, as it is the most frequent and widely distributed species of Cortinarius in the Southern hemisphere (Argentina, Chile and New Zealand). However, our knowledge on Patagonian Cortinarius diversity is still poor and mainly based on Moser & Horak’s work from the early 1960ties. Therefore, we carried out an extensive sampling of Cortinarius species growing in Argentinian Nothofagaceae forests during March-May 2017. Twelve collections representing C. magellanicus based on classical morpho-anatomical characters were used for a phylogenetic analysis based on rDNA ITS sequences. Sequences from Cortinarius Typus material and closely related sequences from GenBank were also included in the study. Our results showed that Cortinarius magellanicus represents a complex of species composed of at least 4 phylogenetic lineages, each with strong regionalism and distinct host associations. This complex is closely related to C. janthinophaeus, with has dry pilei and stipe, thus being a typical representative of the subgenus Telamonia. This highlights that, especially in Southern hemisphere, many groups of Cortinarius have the tendency to form slimy layers on pileus and stipe. Thus, the subgenus Myxacium is paraphyletic and artificial. Cortinarius magellanicus Spegazzini is re-defined and described based on the neotype material. Moreover, C. magellanicoolbus and C. roblerauli are proposed as new species based on consistent morph-anatomical characters and a typical ecology. The clear definition of taxa in the C. magellanicus species complex is of high relevance, given the abundance of these fungi, their ectomycorrhizal role in Nothofagaceae forests and the fact they are regularly collected and consumed.

Notes
1.6

Effect of deletion *gad1* and *gad2* genes on growth and development of *Neurospora crassa*

Daniela Kasáková, Svetlana Kryštofová

Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovak.

Glutamate decarboxylase is the key enzyme for synthetizing γ-aminobutyric acid from glutamate in the GABA shunt. For this work was used *Neurospora crassa* as model organism, which genome encodes two genes for glutamate decarboxylase, *gad1* and *gad2*. The main objective of this work was expression profile of both glutamate decarboxylase genes and phenotypic and metabolomic analysis of GABA shunt genes in *N. crassa*. Transcriptomic analysis revealed significant differences between *gad1* and *gad2* expressions, but phenotypic analysis did not uncover any notable differences between the wild type and *gad1* defective strain. Metabolomic analysis carried out by NMR spectroscopy determined many significant alterations in various metabolites and intermediates in strains defective in GABA metabolism.

Notes
2.1

_trichoderma viride_ bio-waste pre-treatment for enhanced biogas production

Rudolf Markt, Mira Mutschlechner, Paul Illmer, and Andreas Otto Wagner

University of Innsbruck, Institute of Microbiology, Technikerstraße 25d, 6020 Innsbruck, Austria.

As fossil fuels are expected to become less abundant, more expensive, and of increasing environmental concern, there is a current global need for clean and renewable energy sources. In this regard, bioconversion of (ligno)cellulosic biomass via anaerobic digestion provides considerable potential since it represents one of the most cost- and energy-efficient techniques. However, successfully overcoming the inherent recalcitrance of (ligno)cellulose is of utmost importance to obtain high yields and productivity indispensable to commercial success. Improvements therefore rely on effective pretreatment methods that alter and/or remove structural impediments, thus facilitating the accessibility and digestibility of (ligno)cellulosic substrates during fermentation.

The already developed [1,2], but not yet sufficiently understood process of biological pre-treatment using _Trichoderma viride_ represents a cost- and energy efficient technique using available resources. In the presented project biomass pretreatment is carried out by using _Trichoderma viride_ in an aerobic upstream process prior to anaerobic digestion. The experimental setup is therefore divided into two parts: (i) aerobic pre-treatment and (ii) subsequent anaerobic digestion of pre-treated samples. During aerobic pretreatment fungal spores are used for inoculation, whereas an equivalent volume of inactivated spores is added to the control approaches. With the purpose of evaluating the impact of pre-treatment on the subsequent anaerobic digestion, single parameter variation studies will be performed and optimized for maximum methane yield. Since this process, once understood in detail, has the potential to increase middle to large scale biogas production, pretreatment contributes to a sustainable energy use in the future. However, aim of the present project is not the development of a novel pretreatment technique for middle or large scale application but to gain further insights into the complex biochemical and microbiological processes involved in pretreatment and concomitant biogas enhancement.

Therefore the project aims are:

- to investigate the effect of _T. viride_, its distribution in the substrate and cellulolytic activity during the aerobic pre-treatment,
- to investigate _T. viride_ triggered biochemical changes during the aerobic pre-treatment in order to understand the impact on the downstream anaerobic digestion process, and
- to investigate the engaged fungal and bacterial consortia during aerobic pre-treatment as well as the bacterial and archaean community during downstream anaerobic digestion.


2.2

Resistance screening of commonly used antifungals in rare yeasts

Antonio Pérez-Hansen¹, Michaela Lackner¹ and Cornelia Lass-Flörl¹

¹Division of Hygiene and Medical Microbiology, Innsbruck Medical University, Austria.

Infection by Candida spp. is one of the most common nosocomial infections in the world and is the cause of death of millions of patients every year. In this project we have focused on rare Candida species with special interest in C. rugosa, C. inconspicua and C. ciferii. These species are responsible for a very little proportion of the infections, but are associated with a high resistance level and high mortality rate. Since rare, no comprehensive study have been made and epidemiological cut off values are missing.

In this study we tested a collection of 266 suspected rare strains. Susceptibility was tested against the most commonly used antifungal drugs using different methods (broth-macrodispilution using modified EUCAST guidelines and commercial E-test). The objective was to see the distribution of every species to each antifungal drug and to try to set characteristic values (MIC50 and MIC90).

As a short conclusion from this study we can say that these rare species tend to have an overall higher resistance level than the more common species such as Candida albicans. The study also shows that echinocandins seems to be a more effective treatment in vitro than the azoles and than amphotericin B. Finally we can also speculate that the mechanisms involved in resistances in some of these species are intrinsic as other phylogenetically closely related species have similar resistance patterns.

Notes
Chemotropic growth of *Trichoderma atroviride* towards host-derived compounds is not affected by deletion of the tmk1 MAPK gene

Dubraska Moreno-Ruiz, Alexander Lichius, Susanne Zeilinger-Migsich

Institute of Microbiology, University of Innsbruck, Innsbruck, Austria.

*Trichoderma atroviride* is a mycoparasitic fungus used as biological control agent against plant-pathogenic fungi. Because sensing and recognition of prey-derived signals are essential for the successful mycoparasitic fungus-fungus interaction, we have established an assay that quantifies the chemotropic response of the mycoparasite towards different chemical compounds and culture supernatants from the three different fungal prey species *Rhizoctonia solani*, *Botrytis cinerea*, and *Sclerotinia sclerotiorum*. In addition to the *T. atroviride* wild type strain P1, the Δtmk1 gene deletion mutant lacking the terminal MAP kinase of the highly conserved pheromone response pathway was included in our analysis. Previous studies indicated an involvement of Tmk1 orthologues in filamentous growth conidiogenesis and chemotropism, hence we wanted to corroborate its importance for mycoparasitism.

Culture supernatants were produced by culturing *T. atroviride* in the presence of purified prey cell walls and were then used as localised substrate in chemotropism assays performed on minimal medium comparing the responses of conidial germlings and microcolonies of *T. atroviride* wild type and Δtmk1 mutant, respectively. The culture supernatants elicited similar positive chemotropic response in both strains. However, chemicals promoting negative chemotropism, including copper sulfate pentahydrate, manganese chloride, and zinc sulfate heptahydrate, triggered a higher chemotropic index in the mutant strain compared to the wild type. Also, the amount of bipolar germlings was lower in the mutant upon interaction with chemoattractant supernatants, as well as in the presence of chemorepellant chemicals. Interestingly, in microcolony confrontation assays, the host fungus *R. solani* displayed much more pronounced evasive growth when challenged with Δtmk1 compared to the wild type control, a feature that interestingly was not observed in the interactions with either *B. cinerea* or *S. sclerotiorum*.

Together, these findings indicate that the deletion of Tmk1 does not affect the positive chemotropic behavior of *T. atroviride* towards host signals. The apparent increased growth inhibition of *R. solani* is an interesting observation that we attribute to the constitutively increased secretion of mycoparasitic compounds and secondary metabolites. How this phenotype correlates to the apparently negatively affected recognition of potential toxic compounds is currently unclear.

Notes
An improved CRIB reporter enables the identification of individual hyphae of *Trichoderma atroviride* engaged in mycoparasitic attack

Alexander Lichius, Linda Salzmann, Dubraska Moreno-Ruiz & Susanne Zeilinger

Institute of Microbiology, University of Innsbruck, Innsbruck, Austria.

**Background:** Launching a mycoparasitic attack depends on the perception of host-derived signals by individual hyphae of the mycoparasite. However, not all hyphae constituting the colony periphery execute the same cellular programs at the same time. Therefore, a principal difficulty for researching host-parasite interactions on the cellular and molecular level is the identification of individual hyphae that at the time of observation are actively engaged in mycoparasitic signalling. This becomes specifically important for single-molecule imaging techniques where pinpointing such active hyphae is a prerequisite for efficient data acquisition.

**Objectives:** Our rational was to establish a fluorescently-labelled CRIB reporter to visualise those peripheral hyphae of the mycoparasite *Trichoderma atroviride* that grow positively chemotropic towards a host fungus, and thus most likely execute mycoparasitic cellular programs.

**Methods:** Fluorescent CRIB reporters exclusively associate with active, i.e. GTP-bound, Cdc42 and Rac1 GTPases, and specifically label hyphae that show active polarised tip growth. Using site-directed mutagenesis we generated an EGFP variant with 20-fold increased brightness which significantly improved CRIB detection. To allow a clear distinction between parasite and host hyphae in the contact zone we used as confrontation partner and prey *Botrytis cinerea* expressing cytoplasmic mCherry.

**Conclusions:** The novel *T. atroviride* CRIB-mbasicGFP reporter localises inside the Spitzenkörper and as apical crescent/cap in growing hyphae. Repositioning of apical GTPase activity precedes and thus regulates tip growth directionality. Hence, the analysis of CRIB reporter dynamics allows differentiation and prediction of positively chemotropic hyphae, and therefore enables a quantitative approach on mycoparasitic signaling in relation to host-parasite distance and different host species.

---

Notes
Is a lipoxygenase involved in 6-Pentyl-α-Pyrone biosynthesis in Trichoderma atroviride?

Verena Speckbacher; Rainer Schuhmacher; Susanne Zeilinger-Migsich

1Institute of Microbiology, University of Innsbruck, Innsbruck, Austria.
2Analytikzentrum IFA Tulln, Tulln an der Donau, Austria.

Trichoderma atroviride is a mycoparasitic fungus able to antagonize fungal plant pathogens. Because of these properties, T. atroviride finds application in agriculture as biological control agent. Secondary metabolites are among the main agents determining the strength and progress of the mycoparasitic attack. The main secondary metabolite produced by T. atroviride is 6-pentyl-α-pyrone (6-PP), an unsaturated lactone derived from fatty acid metabolism that exhibits anti-fungal activity and plant growth promoting characteristics. Despite these biocontrol-associated bioactivities of 6-PP, the pathway underlying its biosynthesis still awaits clarification.

Based on isotopic labelling experiments, the oxidation of linoleic acid by a lipoxygenase (lox) was proposed as the first step in 6-PP biosynthesis. Accordingly, 6-PP producing species such as T. atroviride, T. gamsii and T. harzianum encode a lox gene (lox1) in their genomes while non-producers such as T. virens and T. reesei do not. In T. atroviride, lox1 furthermore is upregulated during the mycoparasitic interaction with Rhizoctonia solani, a condition resulting in enhanced 6-PP levels. Based on these evidences indicating a role of lox1 in 6-PP biosynthesis, we generated T. atroviride Δlox1 gene deletion mutants. Phenotypic analyses of Δlox1 mutants revealed increased radial growth compared to the parental strain. However, lox1 gene deletion did not affect the mycoparasitic and antifungal activities of T. atroviride and GC-MS based quantification of secreted 6-PP levels revealed similar quantities produced by the mutants and the wild-type. We hence conclude that lox1 affects radial growth of T. atroviride but is not involved in the biosynthesis of 6-PP. Experiments analyzing a putative role of lox1 in the interaction of T. atroviride with plants such as priming of the induced systemic resistance (ISR) response are in preparation.

Notes
TOR kinase signaling in the mycoparasite *Trichoderma atroviride*

Hoda Bazafkan, Rossana Segreto, Martina Schenk, Susanne Zeilinger

Institute of Microbiology, University of Innsbruck, Innsbruck, Austria.

The filamentous fungus *Trichoderma atroviride* has the ability to antagonize and control a wide range of plant-pathogenic fungi. Yet despite its long-term application as a biocontrol agent, the molecular mechanisms governing its mycoparasitic activity are poorly understood. Quality and quantity of the available nitrogen source are important determinants of growth and mycoparasitism. In this respect, the TOR (target-of-rapamycin) kinase plays a fundamental role in orchestrating nutrient sensing, growth and metabolism. The objective of this study is to decipher the function of proteins putatively involved in the TOR signaling pathway in regulation of nitrogen response and mycoparasitism. To this end, deletion mutants of *tsc1, tsc2, are1, rhe2* and *npr1* were generated by replacing their open reading frames (ORF) with a hygromycin resistance cassette. Considering that *tor1* – encoding the central kinase of the pathway - is an essential gene and stable deletion mutants are not feasible, conditional *tor1* silencing under the control of a Tet-On expression system is being developed. The *T. atroviride* wild type and all deletion mutants except Δ*are1* showed decreased radial growth in the presence of the TOR kinase inhibitor rapamycin. Growth of the mutants on the poor nitrogen source urea was concentration-dependent and improved at higher concentrations. Interestingly, Δ*rhe2* and Δ*npr1* showed improved radial growth on urea compared to ammonium. Moreover, we observed production of yet uncharacterized brown-colored droplets in all strains except for Δ*are1* and Δ*tsc1* exclusively in the presence of 10 mM urea indicating exudate production. Our data provide the first evidence for an important role of the TOR pathway in nitrogen signaling in *T. atroviride*.

Notes
3.1

Searching for (photosensitive) bioactive fungal metabolites via a modified antimicrobial EUCAST susceptibility test

Desirée J. Artmann\textsuperscript{1}, Pamela Vrabl\textsuperscript{1}, Bianka Siewert\textsuperscript{2} and Wolfgang Burgstaller\textsuperscript{1}

\textsuperscript{1}Institute of Microbiology, University of Innsbruck, 6020 Innsbruck, Austria
\textsuperscript{2}Institute of Pharmacy, Department of Pharmacognosy, University of Innsbruck, 6020 Innsbruck, Austria

Due to the steep rise of antibiotic resistance, there is an urgent need to find novel bioactive metabolites [1]. Recent research renewed the interest in microorganism-derived natural products as it became apparent that their potential as source of bioactive compounds has been vastly underestimated [1, 2]. While there exist standardized susceptibility testing methods for antimicrobial agents such as EUCAST [3, 4] priorisation methods to screen for highly bioactive fungal extracts are still vastly unstandardized.

Aim of this work was to establish a priorisation assay for fungal extracts based on a modified EUCAST susceptibility test. This method should allow on the one hand screening for bioactive compounds against gram-positive bacteria, gram-negative bacteria and yeasts. In addition, this prescreening method should be designed to screen for photosensitive metabolites, which could be used for phototherapeutic applications.

The prescreening tests with different fungal extracts (aceton extract, methanol extract and petrolether extract from various ascomycetes and basidiomycetes) were performed in liquid medium in sterile 96-well microtiter plates using Mueller-Hinton broth [4] or RPMI-1640 2 % G [3] as media. After incubation (24 hours for bacteria, 48 hours for yeasts, 30 °C) and subsequent photometrical analysis at 530 nm, growth in all fungal extracts was compared to the growth control. Bioactive fungal extracts were then selected and further analysed via LC-MS to prioritize metabolites of interest.

The arrangement of the samples on the 96-well microtiter plate was furthermore designed in such a way to allow for screening of photosensitive metabolites, which has previously been established for cancer cells [5, 6]. To test for photosensitivity, which has yet to be begun, the cultures will be exposed to light with defined wavelengths. The poster highlights some examples of successful priorisations of various fungal extracts and presents our current efforts to establish a prescreening method for photosensitive metabolites.

\[6\] Rixel et al. (2016). Chem Sci 7: 4922-4929
3.2

Primary Succession of Active Fungal Communities in a Glacier Foreland

P. Dresch, U. Peintner

Institute of Microbiology, University of Innsbruck, Innsbruck, Austria.

Retreating glaciers provide a suitable way to study ecological succession in a space-for-time substitution. In this setting, we investigate the change in active fungal communities along the early stages of soil formation (2-20 years free of ice).

In-growth mesh bags filled with sterile quartz sand were buried in five plots, each, at three sampling sites in the glacier foreland. Active, hyphae-forming fungi grow through the soil, randomly passing through the mesh bags where they leave traces of biomass on the quartz sand. Upon retrieval, DNA was extracted from the filling, and amplicon-based metagenomics were used to identify the active fungal biota, namely fungi which have been growing in during the time of incubation. In-growth mesh bags were incubated in soil both during summer and winter to assess seasonal differences in the active soil fungal communities along the gradient. Further, DNA was extracted from soil samples and analyzed in parallel to allow for evaluation of the mesh bag approach.

The combination of mesh-bag with metagenomics aims to circumvent tedious isolation procedures or use of RNA-based methods for detecting actively growing fungi, making it fast and cost-efficient.

The study shows that fungal communities do already differ significantly between the early stages of soil development and an increase in species richness was observed along the succession. The seasonal differences (summer vs. winter) in fungal community composition are quite distinct on bare ground, however, they become less prominent as soil formation proceeds. The comparison of mesh bags to soil samples did not show significant differences.

Notes
The Aspergillus fumigatus leucine biosynthesis-regulator LeuB is crucial for adaptation to iron starvation and virulence in Galleria mellonella

Thomas Orasch¹, Sophie Pleifer¹, Ulrike Binder², Fabio Gsaller¹, Michael Bromley³, Hubertus Haas¹

¹Department of Molecular Biology, Medical University of Innsbruck, Austria.
²Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Austria.
³The University of Manchester, Manchester Academic Health Science Centre, Manchester, UK. The Manchester Fungal Infection Group, The University of Manchester, Manchester, UK.

The mould Aspergillus fumigatus is the most common airborne fungal pathogen of humans causing allergic reactions and severe invasive diseases in immunocompromised patients. In order to identify potential novel targets for antifungal therapy, we investigated the mechanisms involved in biosynthesis and regulation of the amino acid leucine, which represents an essential amino acid for humans. Therefore, we generated three A. fumigatus mutant strains lacking either the leucine biosynthetic enzymes LeuA (α-isopropylmalate isomerase, Afu2g11260) or LeuC (isopropylmalate synthase, Afu1g15000), or the leucine regulatory transcription factor LeuB (Afu2g03460). Deficiency in either LeuA (strain ΔleuA) or LeuC (strain ΔleuC) resulted in leucine auxotrophy, whereby the ΔleuC mutant required significantly higher leucine supplementation for growth than the ΔleuA mutant. Deficiency in LeuB (strain ΔleuB) resulted in partial leucine auxotrophy, i.e. the mutant was able to grow without leucine supplementation but required leucine supplementation for full growth. Interestingly, the ΔleuB mutant displayed significantly decreased resistance to iron starvation. In the Galleria mellonella infection model, deficiency of LeuA, LeuB and particularly LeuC attenuated virulence of A. fumigatus. In conclusion, these data demonstrate that leucine metabolism is a virulence determinant of A. fumigatus and reveal an unprecedented crosstalk between leucine and iron metabolism.

Notes
3.4 Identification and characterization of antifungal drug targets in *Aspergillus fumigatus*

Anna-Maria Dietl¹, Jorge Amich², Sixto Leal³, Ulrike Binder⁴, Nir Osherov⁶, Hubertus Haas²

¹Division of Molecular Biology, Medical University of Innsbruck, Austria.
²Medical University Würzburg, Germany, ³Department of Human Microbiology, Sackler School of Medicine, Tel Aviv, Israel.

*Aspergillus fumigatus* is the most prevalent airborne fungal pathogen causing invasive fungal infections in immunosuppressed individuals. Limitations in antifungal therapy arise from poor diagnostics and limited options for treatment. In order to identify novel potential targets for development of antifungal drugs, we generated auxotrophic mutant strains lacking primary metabolic pathways that are not present in mammalia, e.g. biosynthesis of essential amino acids and vitamins. Genes were replaced by a hygromycin resistance cassette (Δ strains) and to confirm gene deletion-specific effects, mutant strains were complemented (c strains) with the respective functional gene copy. For phenotypic analysis, 10⁴ conidia were point-inoculated on various growth media and incubated at 37° for 48 hours. Virulence was assayed in different infection models. We identified four essential biosynthetic pathways in *A. fumigatus*: (i) biosynthesis of the amino acid histidine, (ii) biosynthesis of the vitamin riboflavin and (iii) biosynthesis of the vitamin pantothenic acid and (iv) biosynthesis of the cofactor siroheme, which is essential for sulfate and nitrate assimilation. Pathways (i-iii) were identified to play an essential role in virulence and represent novel attractive targets for improvement of antifungal therapy.

---

Notes
3.5

The bZIP transcription factor HapX is regulated at multiple levels to control iron homeostasis in *Aspergillus fumigatus*

Manuel S. López-Berges and Hubertus Haas

Division of Molecular Biology, Medical University of Innsbruck, Austria.

HapX is a bZIP transcription factor required for the regulation of different mechanisms aimed to control iron homeostasis in filamentous fungi. Importantly, in the opportunistic human pathogen *Aspergillus fumigatus*, HapX-mediated fungal adaptation to iron-limiting conditions is essential for virulence; therefore, and because of the differences in iron regulation between mammals and fungi, its study might serve to improve therapy and diagnosis of fungal infections. It has long been known that hapX is repressed by iron at the transcriptional level, partially dependent on the GATA factor SreA, but nothing is known about other ways of regulation. Here we show that HapX protein stability is very low and decreases dramatically during a shift from iron depleted to iron-replete conditions, while siderophore biosynthetic enzymes appear to be quite stable. Importantly, we confirmed that this post-translational mechanism depends on the phosphorylation status of the HapX protein. Additionally, we have identified a new regulatory mechanism indicating that iron also controls the stability of hapX mRNA. Taken together, our results indicate that regulation of HapX is crucial to adapt from iron depleted to iron-replete conditions in *Aspergillus fumigatus*.

Notes
Bioluminescent *Mucor circinelloides* – a promising new tool to study mucormycosis and antifungal drug efficacy

**U Binder, MI Navarro-Mendoza², FE Nicolas², C Lass-Flörl¹ and V Garre²**

¹Division of Hygiene and Medical Microbiology, Medical University Innsbruck, Austria  
²Department of Genetics and Microbiology, Faculty of Biology, University of Murcia, Murcia, Spain

**Objectives.** Invasive infections caused by members of the *Mucorales* (mucormycosis) have increased in the last years, making it the third most common invasive fungal infection after aspergillosis and candidiasis. Despite this increasing clinical relevance, little is known about the establishment of disease, its progression and successful therapy. New tools to study this disease in more detail are needed, therefore the objective of this work was to construct a luciferase expressing *Mucor circinelloides* strain, as one representative of mucormycosis causing pathogens. Here, we describe the construction and functional analysis of the strains, which will further be used as a reporter system for *in vivo* and *in vitro* models of Mucorales infections.

**Methods.** A leucine auxotroph *M. circinelloides* strain, R7B, was used as recipient strain to allow selection of transformants on selective medium. Firefly luciferase gene without the peroxisomal target sequence was cloned in the pMAT1477 vector under the control of a constitutive promoter. Linear plasmid was used to transfect *M. circinelloides* protoplasts. The targeted integration of the whole construct in the *carRP* gene resulted in easy identification of transformants, appearing as white colonies. Homokaryons were obtained by sequential plating on selective media and checked for light emission under various conditions in *in vitro* assays.

**Results.** Expression of firefly luciferase was successful in *M. circinelloides* at several conditions and light emission was detectable by imaging and with a luminometer. Data so far indicate the strain being suitable for further *in vivo* and *in vitro* studies. Phenotype, virulence potential and antifungal susceptibility are currently compared to wild-type strains.

**Conclusion.** The construction of this first bioluminescent *Mucor* strain will allow for the visualization of temporal and spatial progression of infection by a non-invasive method in insect and murine models, and the testing of antifungal efficacy by other means than survival only. This will give valuable new insights in the pathogenesis of Mucorales infections.

---

**Notes**
4.1

AN4022 – A novel HDAC complex component as basis for antifungal therapy

Silke Gross¹, Leopold Kremser², Herbert Lindner², Stefan Graessler¹, and Ingo Bauer¹

¹Division of Molecular Biology, Biocenter, Medical University of Innsbruck
²Division of Clinical Biochemistry, Biocenter, Medical University of Innsbruck

Background. An efficient adaptation of opportunistic pathogenic fungi to the host environment is crucial for a successful establishment of infection. Distinct histone modifying enzymes like histone deacetylases (HDACs) are important factors for a proper regulation of genes required for such adaptation processes. RPD3-type HDACs of filamentous fungi exhibit a C-terminal extension that is not found in other eukaryotes and is indispensable for fungal growth and development. Since RPD3-type HDACs exert their function as protein complexes, such sequence peculiarities might represent important binding sites for novel complex partners that in turn might serve as promising targets for novel antifungal compounds. Previously, we were able to identify an uncharacterized conserved fungal protein (AN4022) being part of Aspergillus nidulans RpdA complexes. Orthologs of AN4022 can exclusively be found in Eurotiomycetidae, including a number of important fungal pathogens such as A. fumigatus, A. terreus, A. flavus, Penicillium marneffei, Coccidioides immitis, or Histoplasma capsulatum, indicating unique function in this fungal taxon.

Methods/Results. To characterize the role of AN4022 in fungal growth and development, deletion mutants and complemented strains as well as strains expressing Venus- or TAP-tagged AN4022 were generated. Moreover, for a comparative analysis of effects caused by the disruption of an RPD3 complex partner common in all eukaryotes, SntB, was also deleted in A. nidulans. Mutant strains were subjected to phenotypic analysis under different growth and stress conditions. Preliminary results indicate reduced growth and sporulation and higher susceptibility to osmotic and heat stress of both mutants, though these effects are more pronounced in the sntB mutant. We further used TAP-tagged AN4022 for affinity purification and were able to identify an additional protein AN8823 as novel RpdA complex component together with AN4022 in A. nidulans. Currently ∆AN8823 and ∆AN4022/AN8823 double mutant strains are being generated.

Conclusion. We propose that increased susceptibility of AN4022 and sntB mutant strains against different stressors might be important during infection since functional stress response pathways are known to be essential for full virulence. Coming experiments will include further phenotypic analysis under oxidative stress in order to select proper growth conditions for subsequent transcriptome analysis. Further, strains expressing TAP-tagged RpdA complex partners will enable determination of size and in vitro activity of distinct HDAC complexes.

Notes
Transcriptomic interaction during clubroot disease development

Stefan Ciaghi\(^1\), Arne Schwelm\(^{1,2}\), Sigrid Neuhauser\(^1\)

\(^1\)University of Innsbruck, Institute of Microbiology, Technikerstraße 25, 6020 Innsbruck, Austria.
\(^2\)Swedish University of Agricultural Sciences, Department of Plant Biology, Uppsala BioCenter, Linnean Centre for Plant Biology, P.O. Box 7080, SE-75007 Uppsala, Sweden.

*Plasmodiophora brassicae*, the causal agent of clubroot disease, is responsible for reduced yields of brassica crops worldwide. Use of pesticides, fungicides or antibiotics has no effects, as *P. brassicae* is a protist belonging to the eukaryotic super-group of Rhizaria. Therefore, a better understanding of how this protist interacts with its host plants is of fundamental need. Recently published genomes of *P. brassicae* and a number of studies on host transcriptomic response to clubroot are becoming available. However, all these studies focus either on the host or on the pathogen but not on a combined gene expression analyses of both, the pathogen and the host. Here we investigated the transcriptomic interaction of *P. brassicae* and kohlrabi (*Brassica oleracea* var. *gongylodes*) from field samples in Ranggen, Tyrol, Austria. Host response differs across roots of individual, infected plants. Some roots develop clubroot symptoms and others do not. In infested roots, classical plant defence mechanisms were downregulated, whereas in symptomless roots many defence response genes were significantly upregulated. Functional annotation of about 50% of the *P. brassicae* sequences was possible. Some of the highest expressed *P. brassicae* genes in club roots were a salicylic acid methyl transferase, a predicted glutathione-S-transferase, and predicted ankyrin repeat domain-containing proteins. However, the challenge when analysing the interplay of host and parasite remains the high number of genes with unknown function, particularly the ones with high expression levels.

---

Notes
4.3

Studying phytomyxid-host interactions with in-situ monitoring of gene expression

Julia Badstöber, Martin Kirchmair, Sigrid Neuhauser

University of Innsbruck, Institute of Microbiology, Technikerstraße 25, 6020 Innsbruck, Austria.
E-mail address: julia.badstoeber@uibk.ac.at

Plant-pathogen interactions often follow spatial and temporal developmental dynamics where the expression of genes in both, the pathogen and the host undergo fundamental changes. Therefore, it is of great interest to find, detect, and localize the stages where genes are active, to understand these processes and to break down the interactions to individual cells and the whole plant.

We used a rolling-circle-amplification (RCA) FISH and single-molecule (sm) FISH method to monitor the presence of mRNAs at the single-cell level in a phytomyxid-host pathosystem. The fundamental technique for RCA-FISH is based on three specific designed and shaped probes, whereas smFISH is based on a series of 48 fluorescently labelled oligonucleotides.

With these methods we could detect the expression of three selected pathogen genes and could link them to specific points of pathogen development. We present the results for a methyltransferase, which is involved in suppressing plant defence mechanisms, a chitin synthase, which is estimated due to chitin-containing spores, and actin as housekeeping gene.

Our study demonstrated that both described methods allow to localise expressed genes along spatiotemporal gradients and to link processes to specific points in time of host-pathogen interactions. These techniques have the potential to rapidly increase our understanding of key processes involved in complex plant-microbe interactions on single cell level.

Notes
4.4

The dynamics of brown algal cell walls during infection with the obligate biotroph protist parasite *Maullinia ectocarpii*

Mohammad Etemadi†, Johannes Pallua‡, Philipp Zelger††, Monika Ritsch-Marthe‡‡, Claire MM Gachon‡‡, Sigrid Neuhauser†

†University of Innsbruck, Institute of Microbiology, Technikerstraße 25, 6020 Innsbruck, Austria
‡Medizinische Universität Innsbruck, Sektion für Allgemeine Pathologie, Müllerstraße 44, 6020 Innsbruck, Austria
††Medizinische Universität Innsbruck, Sektion für Biomedizinische Physik, Müllerstraße 44, 6020 Innsbruck, Austria
‡‡Scottish Association for Marine Science, Scottish Marine Institute, Oban, PA37 1QA, UK
E-mail address: Mohammad.Etemadi-Shalamzari@uibk.ac.at

It is well known that the cell wall is one of the challenging barriers for pathogens to infect their hosts. But, getting through the cell wall and the cell wall modification caused by an infection has two faces: changes made by the parasite often trigger changes made by the host to prevent the parasite to get in. The phytomyxean parasite *Maullinia ectocarpii* infects the filamentous brown algae (*Macrocystis pyrifera*). Upon the settlement of the spores to the host surface, cysts are formed, and their content is injected into the host through the host cell wall by mechanical force. Then the pathogen causes hypertrophy within the host cell cytoplasm. This initiates a remodeling of brown algae cell wall as well. Here we studied the cell wall modification of female gametophytes of *M. pyrifera* during an infection with *M. ectocarpii*. Alginate is the main cell wall component of brown algae and is a polysaccharide which is only found in brown algal cell walls and beyond brown algae is only found in a few bacterial genera. The alginate biosynthesis pathway is not yet fully determined in brown algae, but the final step, the polymerization of the sugar mannuronan to alginate is catalyzed by a gene family called Mannuronan-C5-Epimerases (MC5E). We studied the expression of putative MC5E genes. We demonstrate that among the 28 candidate MC5E genes, five are upregulated, 10 genes downregulated and the rest do not show any significant changes in the interaction with *M. ectocarpii* infection. Moreover, we have shown how MC5E gene family clustered in different groups and their response to this infection. Among the MC5E genes, we have selected some candidates for the further experiments.

Notes
4.5

Platelets as immune players in invasive *Candida* infections

G. Rambach¹, C. Eberl¹, M. Hagleitner¹, M. Hermann², R. Bellmann³, I. Lorenz⁴, M. Ströhle⁴, C. Lass-Flörl¹, C. Speth¹

¹Division of Hygiene and Medical Microbiology, Innsbruck Medical University; Innsbruck, Austria.
²Department of Anesthesiology and Critical Care Medicine, Medical University Innsbruck, Innsbruck, Austria.
³Medical Intensive Care and Emergency Unit, Department of Internal Medicine, Medical University Innsbruck, Innsbruck, Austria.
⁴Department of General and Surgical Intensive Care Medicine, Medical University Innsbruck, Innsbruck, Austria.

Platelets are versatile players of innate immunity. Activation in response to pathogens may lead to multifaceted antimicrobial effects, but also to thrombosis or excessive inflammation. In *Candida* (*C.*) septicaemia, *C.* comes in close contact with platelets with putative subsequent processes such as mutual binding, activation and decrease of viability, which can profoundly influence the clinical outcome; therefore we studied platelet-*Candida*-interactions in vitro as well as in the blood of patients with Candidemia.

In *vitro*, adhesion of platelets to yeast cells and hyphae/pseudohyphae of *C.* was moderate, and only marginal activation of platelets could be demonstrated after co-incubation with clinical isolates of different *C.* species (*albicans, glabrata, parapsilosis, tropicalis, dubliniensis, lusitaniae, rugosa*). The presence of platelets did not affect growth or viability of the fungus. However, in a whole-blood-model we could show strong activation of *C.*-adhered platelets as well as enhanced mutual binding and activation of platelets and neutrophils in the presence of *C.*

Our pilot study revealed that platelets derived from the blood of candidemia patients get significantly stimulated, with enhanced levels of the activation markers CD62P and CD63, increased numbers of circulating platelet-derived microparticles and decrease of platelet numbers (thrombocytopenia). The kinetic of the activation markers and platelet viability in the course of the disease differs between the patients, presumably due to various underlying diseases and drug regimens.

We hypothesize that stimulation of platelets by *Candida* species with subsequent activation of other elements of immunity may improve the outcome of candidemia, but might also harbour the danger of thrombosis and excessive inflammation.

---

Notes
4.6

Galactosaminogalactan secreted from *Aspergillus fumigatus* affects human platelet activity and stimulates complement system

Hemalata Deshmukh¹, Günter Rambach¹, Magdalena Hagleitner¹, Cornelia Lass-Flörl¹, Donald Sheppard², Martin Hermann³, Cornelia Speth¹

¹Division of Hygiene and Medical Microbiology, Medical University of Innsbruck; Innsbruck, Austria
²Division of Infectious Diseases, McGill University Montreal, Canada
³Department of Anesthesiology and Critical Care Medicine, Medical University Innsbruck, Innsbruck, Austria

*Aspergillus* (A.) and mucormycetes species cause severe infections in immunocompromised patients. To understand pathomechanisms and antifungal defence in more detail we studied the role of platelets and complement, which are important innate immunity elements. Recent own studies showed that the secreted fungal polysaccharide galactosaminogalactan (GAG) might be an important player since it affects platelet activity and activates the complement system.

Supernatants (SN) of *Aspergillus* and different mucormycetes isolates were collected after 2 days fungal growth and added to human platelets. GAG secretion, platelet activation and complement deposition on platelets were studied by scanning electron microscopy, FACS, confocal laser microscopy.

Incubation of platelets with *A. fumigatus* and *A. flavus* SN resulted in deposition of secreted fungal material on the platelet surface whereas no deposition was obvious when incubating the platelets with medium and SN of mucormycetes. This deposition of fungal material correlated with expression of GAG by *A. fumigatus* and *A. flavus*. Furthermore, the two SN triggered significant platelet activation. Other GAG effects on the platelets included the deposition of complement factor C3 and the formation of the C5b-9 complex on the platelet surface. A perfect correlation between presence of GAG and platelet activation/opsonization could be underlined by the comparison with different *Aspergillus* and mucormycete species. Furthermore, GAG-induced shedding of microparticles was noticed, which represent important pro-inflammatory mediators in the human body.

Our findings underline the hypothesis that GAG is an important fungal immunomodulatory compound. Putative consequences of its activity include platelet-mediated antifungal attack and support of other elements of the immune network, but also thrombus formation and excessive inflammatory reactions.

---

Notes
<table>
<thead>
<tr>
<th>Name</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artmann, Desirée</td>
<td><a href="mailto:Desiree.Artmann@uibk.ac.at">Desiree.Artmann@uibk.ac.at</a></td>
</tr>
<tr>
<td>Badstöber, Julia</td>
<td><a href="mailto:Julia.Badstoeber@uibk.ac.at">Julia.Badstoeber@uibk.ac.at</a></td>
</tr>
<tr>
<td>Bauer, Ingo</td>
<td><a href="mailto:ingo.bauer@i-med.ac.at">ingo.bauer@i-med.ac.at</a></td>
</tr>
<tr>
<td>Bazafkan, Hoda</td>
<td><a href="mailto:Hoda.Bazafkan@uibk.ac.at">Hoda.Bazafkan@uibk.ac.at</a></td>
</tr>
<tr>
<td>Bilder, Ulrike</td>
<td><a href="mailto:ulrike.binder@i-med.ac.at">ulrike.binder@i-med.ac.at</a></td>
</tr>
<tr>
<td>Burgstaller, Wolfgang</td>
<td><a href="mailto:Wolfgang.Burgstaller@uibk.ac.at">Wolfgang.Burgstaller@uibk.ac.at</a></td>
</tr>
<tr>
<td>Ciaghi, Stefan</td>
<td><a href="mailto:Stefan.Ciaghi@uibk.ac.at">Stefan.Ciaghi@uibk.ac.at</a></td>
</tr>
<tr>
<td>Deshmukh, Hemalata</td>
<td><a href="mailto:hemalata.deshmukh@i-med.ac.at">hemalata.deshmukh@i-med.ac.at</a></td>
</tr>
<tr>
<td>Dietl, Anna Maria</td>
<td><a href="mailto:anna-maria.dietl@i-med.ac.at">anna-maria.dietl@i-med.ac.at</a></td>
</tr>
<tr>
<td>Dresch, Philipp</td>
<td><a href="mailto:Philipp.Dresch@uibk.ac.at">Philipp.Dresch@uibk.ac.at</a></td>
</tr>
<tr>
<td>Etemadi-Shalamzari, Mohammad</td>
<td><a href="mailto:Mohammad.Etemadi-Shalamzari@uibk.ac.at">Mohammad.Etemadi-Shalamzari@uibk.ac.at</a></td>
</tr>
<tr>
<td>Grässe, Stefan</td>
<td><a href="mailto:stefan.graessle@i-med.ac.at">stefan.graessle@i-med.ac.at</a></td>
</tr>
<tr>
<td>Haas, Hubertus</td>
<td><a href="mailto:hubertus.haas@i-med.ac.at">hubertus.haas@i-med.ac.at</a></td>
</tr>
<tr>
<td>Huber, Anna</td>
<td><a href="mailto:A.Huber@i-med.ac.at">A.Huber@i-med.ac.at</a></td>
</tr>
<tr>
<td>Kasáková, Daniela</td>
<td><a href="mailto:kasakovad@gmail.com">kasakovad@gmail.com</a></td>
</tr>
<tr>
<td>Lackner, Michaela</td>
<td><a href="mailto:Michaela.Lackner@i-med.ac.at">Michaela.Lackner@i-med.ac.at</a></td>
</tr>
<tr>
<td>Lichius, Alexander</td>
<td><a href="mailto:Alexander.Lichius@uibk.ac.at">Alexander.Lichius@uibk.ac.at</a></td>
</tr>
<tr>
<td>López-Berges, Manuel</td>
<td><a href="mailto:Manuel.Sanchez@i-med.ac.at">Manuel.Sanchez@i-med.ac.at</a></td>
</tr>
<tr>
<td>Markt, Rudolf</td>
<td><a href="mailto:Rudolf.Markt@uibk.ac.at">Rudolf.Markt@uibk.ac.at</a></td>
</tr>
<tr>
<td>Marx, Florentine</td>
<td><a href="mailto:florentine.marx@i-med.ac.at">florentine.marx@i-med.ac.at</a></td>
</tr>
<tr>
<td>Moreno-Ruiz, Dubraska</td>
<td><a href="mailto:Dubraska.Moreno-Ruiz@uibk.ac.at">Dubraska.Moreno-Ruiz@uibk.ac.at</a></td>
</tr>
<tr>
<td>Nagler, Magdalena</td>
<td><a href="mailto:Magdalena.Nagler@uibk.ac.at">Magdalena.Nagler@uibk.ac.at</a></td>
</tr>
<tr>
<td>Neuhauser, Sigrid</td>
<td><a href="mailto:Sigrid.Neuhauser@uibk.ac.at">Sigrid.Neuhauser@uibk.ac.at</a></td>
</tr>
<tr>
<td>Orasch, Thomas</td>
<td><a href="mailto:thomas.orasch@i-med.ac.at">thomas.orasch@i-med.ac.at</a></td>
</tr>
<tr>
<td>Peintner, Ursula</td>
<td><a href="mailto:Ursula.Peintner@uibk.ac.at">Ursula.Peintner@uibk.ac.at</a></td>
</tr>
<tr>
<td>Pérez-Hansen, Antonio</td>
<td><a href="mailto:Antonio.Hansen@i-med.ac.at">Antonio.Hansen@i-med.ac.at</a></td>
</tr>
<tr>
<td>Rambach, Günter</td>
<td><a href="mailto:guenter.rambach@i-med.ac.at">guenter.rambach@i-med.ac.at</a></td>
</tr>
<tr>
<td>Salgado-Salomón, María Eugenia</td>
<td><a href="mailto:mesalgadosalomon@ciefap.org.ar">mesalgadosalomon@ciefap.org.ar</a></td>
</tr>
<tr>
<td>Siewert, Bianka</td>
<td><a href="mailto:bianka.siewert@uibk.ac.at">bianka.siewert@uibk.ac.at</a></td>
</tr>
<tr>
<td>Sonderegger, Christoph</td>
<td><a href="mailto:christoph.sonderegger@i-med.ac.at">christoph.sonderegger@i-med.ac.at</a></td>
</tr>
<tr>
<td>Speckbacher, Verena</td>
<td><a href="mailto:Verena.Speckbacher@uibk.ac.at">Verena.Speckbacher@uibk.ac.at</a></td>
</tr>
<tr>
<td>Speth, Cornelia</td>
<td><a href="mailto:cornelia.speth@i-med.ac.at">cornelia.speth@i-med.ac.at</a></td>
</tr>
<tr>
<td>Strasser, Hermann</td>
<td><a href="mailto:Hermann.Strasser@uibk.ac.at">Hermann.Strasser@uibk.ac.at</a></td>
</tr>
<tr>
<td>Vrabl, Pamela</td>
<td><a href="mailto:Pamela.Vrabl@uibk.ac.at">Pamela.Vrabl@uibk.ac.at</a></td>
</tr>
<tr>
<td>Wagner, Andreas</td>
<td><a href="mailto:Andreas.Wagner@uibk.ac.at">Andreas.Wagner@uibk.ac.at</a></td>
</tr>
<tr>
<td>Walch, Georg</td>
<td><a href="mailto:Georg.Walch@student.uibk.ac.at">Georg.Walch@student.uibk.ac.at</a></td>
</tr>
<tr>
<td>Zeilinger-Migsich, Susanne</td>
<td><a href="mailto:Susanne.Zeilinger@uibk.ac.at">Susanne.Zeilinger@uibk.ac.at</a></td>
</tr>
<tr>
<td>Zottele, Maria</td>
<td><a href="mailto:Maria.Zottele@uibk.ac.at">Maria.Zottele@uibk.ac.at</a></td>
</tr>
</tbody>
</table>
Thanks to our Sponsors

www.analytik-jena.de

www.biozym.com

www.gluckspilze.com

www.gml.at

www.microsynth.ch

www.mykon.at

www.starkenberger.at