26TH SEPTEMBER 2024

4TH MYCOLOGY TYROL SYMPOSIUM

2024

WELCOME

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MEDIZINISCHE UNIVERSITÄT INNSBRUCK

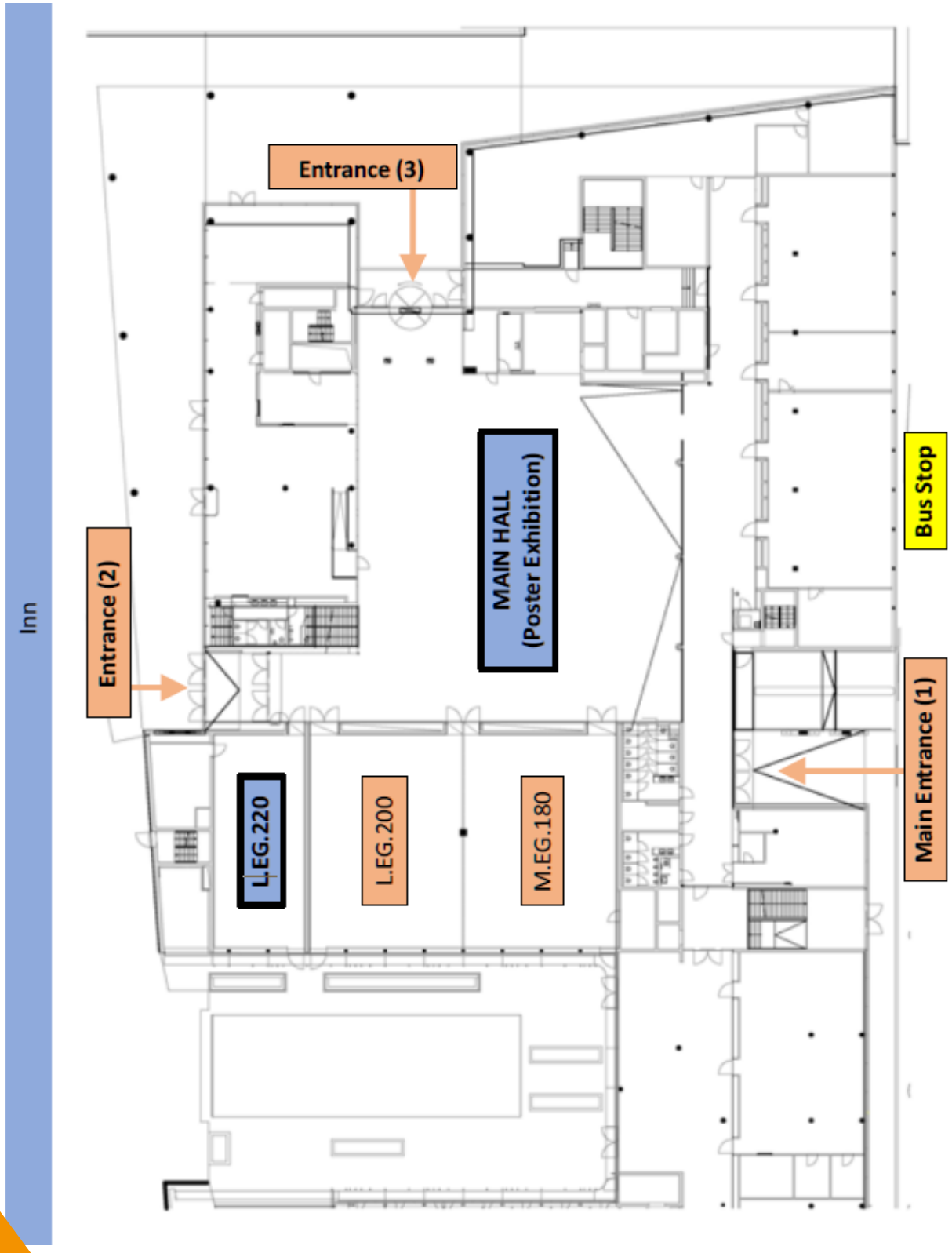
The ***4th Mycology Tyrol Mini-Symposium*** will create a highly interdisciplinary scientific exchange and discussion platform on topics including Molecular Fungal Cell Biology, Fungal Physiology, Environmental Mycology, Microbial Resource Management, Clinical Mycology and Fungal Biocontrol. Researchers from the **Medical University of Innsbruck** and the **University of Innsbruck** will join forces to present their latest advancements in these fields and discuss novel trends. In addition to plenary sessions, students and postdoctoral researchers will have the opportunity to present their projects 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.

Your organization Team

Floor Plan (Ground floor CCB)

The *4th Mycology Tyrol Mini-Symposium* will take place in the ground floor of the CCB in **L.EG.220** (talks) and the **Main Hall** (poster presentations / networking).



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Clara Baldin (University of Innsbruck – Department of Microbiology)

Ingo Bauer (Medical University of Innsbruck – Institute of Molecular Biology)

Stephanie Töpfer (Medical University of Innsbruck – Institute of Hygiene and Medical Microbiology)

Daniel Flatschacher (University of Innsbruck – Department of Microbiology)

Lesley Huymann (University of Innsbruck – Department of Microbiology)

Markus Meitinger (University of Innsbruck – Department of Microbiology)

Program

8:30 - 8:50	**Registration**	
8:50 - 9:00	**Opening remarks**	**by the organizing team**
9:00 - 9:40	**Keynote lecture 1**	**Chaired by Ingo Bauer and Clara Baldin**
9:00	**Joseph Strauss** *BOKU University, Department of Applied Genetics and Cell Biology*	"The internet of fungi – communication and memory within a fungal colony"
9:40 - 10:40	**Plenary session 1**	**Chaired by Ulrike Binder and Lesley Huymann**
9:40	**Daniel Flatschacher** *University of Innsbruck, Department of Microbiology*	"Linking a polyketide synthase gene cluster to 6-pentyl-alpha-pyrone, a *Trichoderma atroviride* metabolite with diverse bioactivities"
10:00	**Ingo Bauer** *Medical University of Innsbruck, Institute of Molecular Biology*	"Online biomass monitoring enables characterization of the growth pattern of *Aspergillus fumigatus* in liquid shake conditions"
10:20	**Cornelia Speth** *Medical University of Innsbruck, Institute of Hygiene and Medical Microbiology*	"The fungus-carcinoma axis: is *Malassezia* able to survive in pancreas and to contribute to Pancreatic Ductal Adenocarcinoma"
10:40 - 11:40	**Coffee break with Poster viewing (session 1 - odd numbers)**	
11:40 - 12:40	**Plenary session 2**	**Chaired by Martin Kirchmair and Markus Meitinger**
11:40	**Ulrike Binder** *Medical University of Innsbruck, Institute of Hygiene and Medical Microbiology*	"Experimental preclinical imaging-compatible animal models of mucormycosis"
12:00	**Markus Neurauter** *University of Innsbruck, Department of Microbiology*	"Exploring Near-Infrared Spectroscopy, Hyperspectral Imaging and MALDI-TOF Mass Spectrometry as novel Characterization Methods for Anaerobic Gut Fungi"
12:20	**Andrea Garvetto** *University of Innsbruck, Department of Microbiology*	"Under the cover of darkness: how clubroot disease affect *Arabidopsis thaliana* physiology during the night"
12:40 - 13:40	**Lunch break and networking**	

13:40 - 14:40	Plenary session 3	Chaired by Susanne Zeilinger and Markus Neurauter
13:40	**Fabio Gsaller** *Medical University of Innsbruck, Institute of Molecular Biology*	"Boosting *Aspergillus* antifungal research combining multiplex CRISPR/Cas9 with counter-selection"
14:00	**Georg Walch** *University of Innsbruck, Department of Microbiology*	"Microbial growth in floor constructions after water damage and artificial suction drying"
14:20	**Sophia Strobl** *University of Innsbruck, Department of Microbiology*	"Exploring the enzymatic potential of Anaerobic Gut Fungi"

14:40 - 15:40	Coffee break with Poster viewing (session 2 - even numbers)

15:40 - 16:40	Plenary session 4	Chaired by Cornelia Speth and Sophie Szedlacsek
15:40	**Mario Gründlinger** *University of Innsbruck, Department of Microbiology*	"Simply cut out – Combining CRISPR/Cas9 RNPs and transiently selected telomere vectors for marker free-gene deletion in *Trichoderma atroviride*"
16:00	**Katharina Russ** *University of Innsbruck, Department of Microbiology*	"Exploring differences in wood decay and pigment patterns among *Serpula lacrymans* strains"
16:20	**Günter Rambach** *Medical University of Innsbruck, Institute of Hygiene and Medical Microbiology*	"The antiseptic N-Chlorotaurine demonstrates its efficacy as inhaled therapeutic in a murine model of *Aspergillus fumigatus* pneumonia"

16:40 - 17:20	Keynote lecture 2	Chaired by Stephanie Töpfer and Clara Baldin
16:40	**Ilse Denise Jacobsen** *Leibniz - HKI, Institute of Microbial Immunology*	" *Candida* colonisation and antibiotic treatment: More than just a risk for candidiasis"

17:20 - 17:40	Closing remarks and Poster prizes	by the organizing team

From 17:40	Open mixer with drinks, snacks and posters

Abstracts – Talks

9:00 - 9:40 **Keynote lecture 1** **Chaired by Ingo Bauer and Clara Baldin**

The internet of fungi – communication and memory within a fungal colony

Harald Berger[1*], Franz Zehetbauer[1*], Markus Bacher[2], Roman Labuda[2], Maria Doppler[3], Florian Kastner[1], Christoph Schüller[1,2,3], and Joseph Strauss [1,2]

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A fungal colony is a branched network of interconnected hyphal cells that exchange cytoplasm, organelles, nutrients and signals through septal pores. An interesting question is, if this cellular network also represents an information network for environmental signals, and if this information may be even stored as a sort of simple cellular "memory" in which a repeated stimulus is recognized quicker and the corresponding response can thus be stronger at the 2nd time of exposure.

We tried to answer these questions using the filamentous ascomycete *Aspergillus nidulans* and its capacity to produce antibacterial compounds as a model system. In our experimental setup we exposed *A. nidulans* on one side of the colony to a bacterial competitor that deprives nutrients and monitored the production of secondary metabolites including defensive compounds all over the fungal colony. This metabolic profiling revealed a spatial restriction of the metabolic responses to certain areas of the colony grown on a solid surface. The type of response depended on the type and place of challenge. We could detect the production of the antibacterial compound orsellinic acid by the fungus only at the site of confrontation with a bacterium which released the starvation-molecule Polaramycin-B[1]. Thus, inhibition of the fungal colony occurred only at the site of bacterial confrontation. So, despite the *A. nidulans* surface-grown colony represents a "cellular network" the information does not freely flow among the connected cells which indicates that the communication and a systemic starvation response between cells through septal pores is spatially limited.

In contrast to the solid surface colony, cells in a hyphal network of properly aerated submerged cultures are far more uniform. This can be seen from e.g., microscopic analyses of fluorescently labelled proteins or by the strong and uniform signals we get from whole-genome transcriptomes (RNA-Seq) or chromatin modification analyses (ChIP-Seq). We used these submerged cultures and indeed found by RNA-Seq a uniform response to the starvation molecule Polaramycin B. Using this

uniform system we now could also ask if repeated exposure to starvation might evoke a sort of memory leading to a quicker and stronger response to a subsequent exposure. Indeed, we found such a transcriptional memory for about 800 genes that responded more vigorously to the 2nd exposure compared to control cultures of the same age, that experienced starvation for the 1st time. Subsequent analysis of the molecular events behind this memory effect identified transcription factor inheritance, certain epigenetic modifications and the production of "metabolic memory molecules" as triggers of fungal cellular memory[2].

[1] H. Berger et al. Polaramycin B, and not physical interaction, is the signal that rewires fungal metabolism in the Streptomyces-Aspergillus interaction. Environ Microbiol. 2022 Oct;24(10):4899-4914. doi: 10.1111/1462-2920.16118.

[2] F. Zehetbauer et al. (2024). Transcriptional memory drives accelerated re-activation of secondary metabolite production in *Aspergillus nidulans* (unpublished).

* these authors contributed equally

Linking a polyketide synthase gene cluster to 6-pentyl-alpha-pyrone, a *Trichoderma atroviride* metabolite with diverse bioactivities

Daniel Flatschacher[1], Alexander Eschlböck[1], Siebe Pierson[1], Ulrike Schreiner[1], Valentina Stock[2], Anna Schöllnast[2], Arne Schiller[2], Veronika Ruzsanyi[2], Susanne Zeilinger[1]

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[2] *Institute for Breath Research, University of Innsbruck, Innsbruck, Austria*

The filamentous fungus *Trichoderma atroviride* is commonly used as a biocontrol agent against crop diseases caused by phytopathogenic fungi due to its high antagonistic activity that includes mycoparasitism and the production of antifungal and antibiotic secondary metabolites (SM). One of these bioactive and mycoparasitism associated SMs is 6-pentyl-α-pyrone (6-PP). We previously showed that 6-PP production in *T. atroviride* reaches its maximum when the fungus is cultivated in darkness or reduced light conditions but is largely inhibited by white light. Despite being one of the most significant SMs produced by *T. atroviride*, the molecular pathway underlying 6-PP biosynthesis has not yet been elucidated.

In this study, we demonstrate that 6-PP is biosynthesized via the polyketide biosynthesis pathway, and identified the PKS core gene essential for its biosynthesis in *T. atroviride*. Using CRISPR/Cas9, we conducted a successful *pks* gene knockout experiment and confirmed the role of this gene in 6-PP biosynthesis via subsequent HPTLC and LC-MS analyses. Deletion of the *pks* gene did not lead to major alterations in mycelial growth or pigmentation but resulted in a significantly reduced inhibitory activity against the plant pathogens *Botrytis cinerea* and *Rhizoctonia solani*. Additionally, we characterized the PKS gene cluster associated with 6-PP biosynthesis at the transcriptomic level, providing valuable insights into the molecular mechanisms underlying this biosynthetic pathway in *T. atroviride*. Finally, a similar pattern of production of 6-PP and 2-pentylfuran was observed which could indicate similarities in the biosynthetic origin and/or physiological role of these two substances.

Online biomass monitoring enables characterization of the growth pattern of *Aspergillus fumigatus* in liquid shake conditions

Ingo Bauer[1], Beate Abt[1], Annie Yap[1], Bernd Leuchtle[2], Hubertus Haas[1]

[1] *Institute of Molecular Biology, Biocenter, Medical University of Innsbruck, Austria*
[2] *SBI, Scientific Bioprocessing, Pittsburgh, USA*

Numerous filamentous fungal species are extensively studied due to their role as model organisms, workhorses in biotechnology, or as pathogens for plants, animals, and humans. Growth studies are mainly carried out on solid media. However, studies concerning gene expression, biochemistry, or metabolism are carried out usually in liquid shake conditions, which do not correspond to the growth pattern on solid media. The reason for this practice is the problem of online growth monitoring of filamentous fungal species, which usually form pellets in liquid shake cultures. Here, we compared the time-consuming and tedious process of dry-weight determination of the mold *Aspergillus fumigatus* with online monitoring of biomass in liquid shake culture by the parallelizable CGQ ("cell growth quantifier"), which implements dynamic biomass determination by backscattered light measurement. The results revealed a strong correlation of CGQ-mediated growth monitoring and classical biomass measurement of *A. fumigatus* grown over a time course. Moreover, CGQ-mediated growth monitoring displayed the difference in growth of *A. fumigatus* in response to the limitation of iron or nitrogen as well as the growth defects of previously reported mutant strains (Δ*hapX*, Δ*srbA*). Furthermore, the frequently used wild-type strain Af293 showed largely decreased and delayed growth in liquid shake cultures compared to other strains (AfS77, A1160p+, AfS35). Taken together, the CGQ allows for robust, automated biomass monitoring of *A. fumigatus* during liquid shake conditions, which largely facilitates the characterization of the growth pattern of filamentous fungal species.

The fungus-carcinoma axis: is *Malassezia* able to survive in pancreas and to contribute to Pancreatic Ductal Adenocarcinoma

Cornelia Speth[1], Nadine Falbesoner[1], Ruben Bellotti[2], Ulrike Binder[1], Cornelia Lass-Flörl[1], Manuel Maglione[2], Günter Rambach[1]

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[2]*Department of Visceral, Transplant, and Thoracic Surgery, Medical University of Innsbruck, Innsbruck, Austria*

Introduction: Recent publications hypothesize that the yeast *Malassezia* can migrate from human gut into the pancreas where it represents a relevant trigger for the progression of pancreatic ductal adenocarcinoma (PDAC). A prerequisite for such an effect is the fungal survival and proliferation in the tumor environment.
Objectives: We aimed: (1) to confirm the augmented presence of *Malassezia* in tumor tissue of PDAC patients; (2) to study the capacity of *Malassezia* to survive and proliferate in the pancreas with its digestive enzymes and in the hypoxic tumor environment.
Materials and Methods: The presence of *Malassezia* in formalin-fixed paraffin-embedded pancreatic cancer tissue was evaluated by PCR analysis. *Malassezia* survival and proliferation in tumor environment were investigated by cultivation in presence/absence of homogenized murine pancreas, human pancreatic juice or under hypoxic conditions.
Results: A small pilot study confirmed the increased presence of *Malassezia* in PDAC tissue compared to tissue samples derived from patients with other pancreatic diseases. The fungal presence was not caused by contamination during pre-surgical interventions such as endoscopic retrograde cholangiopancreatography.
Incubation of *Malassezia* with homogenized murine pancreas or human pancreatic juice demonstrated that the yeast can survive in presence of pancreatic enzymes and even exploit the nutrients for proliferation. This could be confirmed by inoculation of non-homogenized whole murine pancreas with *Malassezia*, followed by fungal staining over time. Hypoxic conditions, which are typical for solid tumor environment, did not interfere with fungal survival.
Conclusion: Confirmation of increased fungal detection in tumor tissue in our study makes a contribution of *Malassezia* to PDAC progression more likely. Furthermore, *Malassezia* is able to survive and grow in pancreatic tumor environment, a prerequisite for its putative role as a pro-carcinogenic trigger.

Experimental preclinical imaging-compatible animal models of mucormycosis

A Resendiz-Sharpe[1], J Scheler[2], C Kandelbauer[2], MI Navarro-Mendoza[3], FE Nicolas[3], I Bauer[4], C Lass-Flörl[2], V Garre[3], G Vande Velde[1] and U Binder[2]

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Introduction
Although infections by mucormycetes are a serious threat in clinical settings, due to fast progression and limited treatment options, little is known about pathogenesis mechanisms.
Aim
Generation of luciferase expressing Mucorales strains to be used for non-invasive monitoring of mucormycosis infection over time in different animal models -especially to establish imaging-compatible preclinical insect and mouse models.
Methods
Codon-optimized firefly luciferase without the peroxisomal target sequence, under the control of two different promoters was cloned into auxotrophic *M. lusitanicus* recipient strains, including gene deletion mutants. Positive, now prototroph, transformants were checked for gene integration by PCR and Southern Blot. Subsequently, growth pattern and light emission under various conditions were determined by luminometer. Selected strains were used for *in vivo* validation in Galleria infection assays and in a neutropenic mouse model and fungal infection was determined by BLI imaging analysis.
Results
Firefly luciferase was successfully expressed in *M. lusitanicus.* upon single integration. Light emission could be measured by luminometer and visualized in animal models. High light signal was obtained in infected *Galleria* larvae 48h after infection but decreased at 96h in those still alive. Likewise, light signals were detected in mice since day 1 post-infection which increased until day 3. On day 4, light signals decreased in tandem with recovery of weight and immunosuppression. Overall, the strains are usable for real-time, non-invasive infection monitoring which could be potentially used for antifungal efficacy assessment by means other than survival.
Conclusion
The successful visualization of *M. lusitanicus* infection by a non-invasive method in insect and murine models, offers new ways to study mucormycosis. Extending this approach to other species will provide valuable insights into the pathogenesis of Mucorales infections.

Exploring Near-Infrared Spectroscopy, Hyperspectral Imaging and MALDI-TOF Mass Spectrometry as novel Characterization Methods for Anaerobic Gut Fungi

Markus Neurauter[1], Julia M. Vinzelj[1], Sophia F.A. Strobl[1], Christoph Kappacher[2], Tobias Schlappack[2], Jovan Badzoka[2], Matthias Rainer[2], Christian W. Huck[2], Sabine M. Podmirseg[1]

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Neocallimastigomycota are a phylum of anaerobic gut fungi (AGF) that inhabit the gastrointestinal tract of herbivores and play a pivotal role in plant matter degradation. Their identification and characterization with marker gene regions has long been hampered due to the high inter- and intra-species length variability in the commonly used fungal marker gene region ITS. While recent research has improved methodology (i.e. switch to LSU D2 as marker region), molecular methods will always introduce bias through nucleic acid extraction or PCR amplification. We explored near-infrared spectroscopy (NIRS), hyperspectral imaging (HSI) and MALDI-TOF mass spectrometry (MALDI) as nucleic acid sequence-independent tools for the characterization and identification of AGF strains. We present a proof-of-concept for all three, achieving an independent prediction accuracy of above 95% for models based on discriminant analysis trained with samples of three different genera. We further demonstrated the robustness of the NIRS and MALDI model by testing it on cultures of different growth age. Overall, NIRS and MALDI provide a simple, reliable, and minimally destructive approach for AGF classification, independent of molecular approaches. The HSI method provides further advantages by requiring less biomass and adding spatial information, a valuable feature if this method is extended to mixed cultures or environmental samples in the future.

Under the cover of darkness: how clubroot disease affect *Arabidopsis thaliana* physiology during the night

Andrea Garvetto, Michaela Hittorf, Freia Benade, Susann Auer, Jutta Ludwig-Müller, Sigrid Neuhauser

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As sessile and light-dependent organisms, plants are inherently attuned to diel cycles of night and day. Among many other processes, diel cycles have been shown to influence the interactions of plants with pathogenic microbes, mostly by means of oscillations in the expression of genes involved in energy allocation and defenses. Specialized plant pathogens evolved to fit host rhythms and/or to act as *zeitgebers*, modifying the plant rhythms to counter defenses, to better tap into host resources and to carve their own niche. Here, for the first time we produced a circadian RNA-seq dataset of the obligate biotrophic parasite *Plasmodiophora brassicae* and its host *Arabidopsis thaliana* in the early (14 days) and late (21 days) phases of clubroot infection. In our first exploratory investigation of this multilayered dataset, we focus on the effects of infection on the plant physiology in daytime and nighttime. We observe a high proportion of genes differentially expressed in the night, which decreases with the host age. GO term analyses show that these genes are mostly enriching processes also influenced during the day, pointing at a previously unrecognized more intense manipulation of the host physiology during the night. Finally, we detect and discuss instances of infection-driven night-specific processes and alterations of night-day rhythms, providing a first temporal atlas of clubroot gene expression.

Boosting *Aspergillus* antifungal research combining multiplex CRISPR/Cas9 with counter-selection

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Aspergillus fumigatus, the main causative agents of invasive aspergillosis, represents one of the deadliest fungal species worldwide and accounts for hundreds of thousands of deaths each year. In addition to a shortage in the antifungal armory comprising only three drug classes, resistance to the major class employed in the clinical setting, the azoles, is steadily increasing. A comprehensive understanding of the molecular mechanisms driving azole resistance is essential for developing new strategies to combat this problem.

The recent discovery of multiple, endogenous counter-selectable markers enabled site-directed insertion of numerous additional expression cassettes into the genome of *A. fumigatus*, which facilitated research applications that required multigene integration. The shortcoming, gene cassettes had to be transformed sequentially.

In this study, we overcame this obstacle by combining the use of CRISPR/Cas9 with the mentioned markers, successfully integrating multiple expression cassettes in a single transformation event. Exploiting this new technique, we generated mutants carrying different resistance alleles and strain-specific fluorescent protein tags to simultaneously analyze four strains during azole treatment employing multicolor fluorescence microscopy.

We anticipate that the presented method will bolster a wide range of research applications that require facile and rapid equipment of strains with multiple expression cassettes and will therefore open new avenues in antifungal research.

Microbial growth in floor constructions after water damage and artificial suction drying

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Indoor growth of microorganisms can become a severe problem after water damage in buildings, and is often localized in floor constructions. Water enters the floor insulation layers between screed and concrete when pipes are clogged or leaking, and microbial growth develops quickly after such events. To avoid this, insulation layers in floor constructions are often dried artificially.
We tested if a routinely used drying method for floor insulation layers based on under-pressure can prevent microbial growth. We built floor constructions, then flooded and dried them. Samples of the insulation layers, either expanded polystyrene (EPS) or artificial mineral fibers (AMF), were taken before flooding, and again after the insulation layers were dried. Densities of fungal and bacterial colony forming units (CFU) were determined by plating suspensions of EPS and AMF samples on appropriate culture media. Pure cultures of bacterial morphotypes were identified by blasting rDNA gene sequences against the NCBI server, while fungi were identified by their morphology.
Before the simulated water damage, *Penicillium* spp. were the most prevalent fungal CFU and spore-forming Bacilliales the most prevalent bacterial CFU in both floor dust, collected on-site before the start of the experiment, and in insulation samples of both types. After water damage and conclusion of the drying procedure, *Acremonium* s.l. were the most prevalent fungi, found in high abundance on both types of insulation material. Spore-forming Bacilliales were still present in high abundances, as well as non-spore forming Bacilliales (Planococcus spp. and Chryseomicrobium spp.) and Actinobacteria. The type of insulation material used in floor construction appeared to have no influence on species composition or abundance of microbial growth. The spatial distribution of fungi and bacteria appeared fully erratic and could not be correlated with residual moisture content, or the sample's location inside the floor construction. Finally, we found that immediate drying of the flooded floor constructions was unable to prevent excessive microbial growth.

Exploring the enzymatic potential of Anaerobic Gut Fungi

Sophia F.A. Strobl[1], Julia Vinzelj[1], Nico Peer[1], Marco Wehner[1], Laura Marte[1], Alex Riederer[1], Eva-Maria Kofler[1], Johannes K. Metzler[1], Pierre Koch[1], Claudio Kalbermatten[2], Julia Veitengruber[3a], Zrinka Raguz Nakic[2], Mathias Effenberger[3b], Christian Ebner[1], Christin Peters[2], Thomas Venus[3b], Veronika Flad[3a], Hans Joachim Nägele[2], Sabine Podmirseg[1]

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Addressing the challenges of waste management and energy production are two central topics of our time, and circular economy may offer solutions to these issues. Biogas plants, for example, can be fueled with agricultural residues to generate energy, however, the effective degradation of these recalcitrant plant materials under anaerobic conditions remains problematic. Anaerobic gut fungi (AGF, phylum Neocallimastigomycota), inhabitants of the gastrointestinal tract of many herbivorous animals, are adept at breaking down plant material rich in lignocellulose by both mechanical action via their rhizoids and enzymatic processes. The outstanding degradation capabilities of AGF, along with their adaptation to mesophilic and anaerobic environmental conditions, position them as a valuable reservoir for enzymes that could enhance breakdown of lignocellulosic biomass (LCB) in biogas plants. In the international project FUNGAS, we aim to elucidate the enzymatic capabilities of AGF and evaluate their applicability to improve biogas production from LCB. Starting with a pre-screening of our long-term cultivated AGF culture collection and freshly isolated strains, enzymes from promising strains will be selected for further evaluation. We will explore three approaches: I) direct implementation of AGF cultures, II) supplementation with enzymes extracted from AGF culture supernatant, III) implementation of recombinantly upscaled enzyme solutions in lab-scale bioreactors and monitoring of the impact on biogas yield and composition. This multi-faceted project aims to develop new ways to generate sustainable energy from LCB.

Simply cut out – Combining CRISPR/Cas ribonucleoprotein (RNP) complexes and transiently selected telomere vectors for marker free-gene deletion in *Trichoderma atroviride*

Mario Gründlinger[1], Chiara Ellensohn[1], Leo Drechsel[1], Ulrike Schreiner[1], Siebe Pierson[1], Clara Baldin[1], Susanne Zeilinger-Migsich[1]

[1]*Department of Microbiology, University of Innsbruck, Innsbruck, Austria*

Trichoderma atroviride is a well-known mycoparasite used to protect plants from fungal pathogens. Expanding the genetic toolbox is essential for facilitating genetic manipulations, such as multiple gene deletions and retransformations in this species. Based on previous studies from *Botrytis cinerea*, we applied the CRISPR/Cas9 system via ribonucleoprotein (RNP) complexes for gene editing and later extended our approach with a transiently stable telomere-containing vector for efficient gene deletion. We deleted three genes: *pks4* (melanin production), *pyr4* (pyrimidine biosynthesis), and *pex5* (shuttling receptor for peroxisomal matrix proteins). The DNA double-strand breaks induced by Cas9 are repaired either by homology-directed repair (HDR) or non-homologous end joining (NHEJ). Unlike in other fungi like *A. fumigatus*, gene deletion using a donor template carrying the hygromycin-resistance gene (hph) with microhomology flanks was less effective in our case. The most successful gene deletion occurred when we co-transformed a temporary telomere vector with the *hph* gene. This vector was rapidly lost after growing cells on non-selective media. At the same time, the cell utilizes the more efficient NHEJ repair mechanism, leading to the loss of the ORF between the two inserted Cas9 cleavage sites. By simply cutting the target DNA with this new approach, we bypassed the need for generating knock-out cassettes and benefit from NHEJ instead of the less efficient HDR. We demonstrate that the combination of CRISPR/Cas9 RNP delivery with transiently stable telomere vectors can also be efficiently used in *T. atroviride* for marker-free gene deletion and vector recycling.

Exploring differences in wood decay and pigment patterns among *Serpula lacrymans* strains

Katharina Russ[1], Susanne Zeilinger-Migsich[1], Martin Kirchmair[1], Sigrid Neuhauser[1]

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One of the most effective brown-rot causing fungi is *Serpula lacrymans*, which colonizes houses in temperate and boreal regions all over the world. In building construction, a house infested with *S. lacrymans* is quite feared, as the removal is very expensive and laborious and has to be done according to official guidelines. *S. lacrymans* spreads very quickly in the houses through vegetative mycelium and through the formation of mycelial cords, which are responsible for the transport of nutrients. As soon as the fungus has established itself on built timber, it begins to degrade cellulose and hemicellulose. We have different *S. lacrymans* strains in culture and are using culture-based methods to determine if these different strains degrade cellulose, starch, pectin and xylan in the same way or not. Additionally, we are conducting wood-decay experiments to assess whether these different strains degrade *Picea abies* wood pieces similarly.
Fungi are known for the ability to produce a wide range of secondary metabolites and *Serpula lacrymans* is no exception. Using HPTLC, we aim to identify whether the different *S. lacrymans* strains show different pigment patterns. To achieve this, we cultivate *S. lacrymans* on minimal media, perform pigment extraction, and then use HPTLC. The HPTLC plates are subsequently derivatized with various spray reagents to visualize amino acids, sugars, phenols, and higher alcohols.

The antiseptic N-Chlorotaurine demonstrates its efficacy as inhaled therapeutic in a murine model of *Aspergillus fumigatus* pneumonia

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Introduction: N-chlorotaurine (NCT) is an excellently tolerated antiseptic substance with broad activity spectrum against pathogens. The well-documented topical applicability recommends inhaled NCT as supportive therapy for fungal infections of the lower airways and was already studied for mucormycetes.
Objectives: We aimed: (1) to evaluate the NCT effectiveness in mouse models of *Aspergillus fumigatus* pneumonia with different immunosuppressive regimens; (2) to compare different NCT concentrations in their capacity to improve the outcome.
Materials and Methods: Mice were immunosuppressed with either cyclophosphamide or cortisone acetate, followed by intranasal inoculation with *A. fumigatus.* Inhalations with 0.1% - 2.0% NCT solution or, as control, sodium chloride three times daily for 10 min started one hour after inoculation and ended after 15 days.
Results: In a subgroup of mice euthanized on day 2, fungi and inflammatory signs were detected in the lungs. In the placebo group, 8/9 or 9/9 mice observed for 15 days died from the infection during this time, while 0/9 to 1/9 died in groups treated with 0.5%, 1.0% and 2.0% NCT ($p < 0.01$ for each concentration versus saline). There was no difference between the two regimens of immunosuppression. With 0.1% NCT, 4/9 mice died ($p = 0.03$ versus the higher NCT-concentrations; $p = 0.0035$ versus control). The fungal load came to 5.28 (4.46; 5.70; median, quartiles) CFU/ml lung homogenate in the control group and to 1.3 (median; maximum 2.45) in the 1% NCT group in mice immunosuppressed with cyclophosphamide ($p = 0.0004$). Values were similar in cortisone groups ($p = 0.0023$). Secondary parameters showed respective significant differences between test and control groups.
Conclusion: Early treatment with inhaled NCT demonstrated highly significant efficacy in improving the outcome of *Aspergillus* pneumonia. A concentration of 1% NCT appears to be optimal, which fits to case experiences with inhalations in humans.

Candida colonisation and antibiotic treatment: More than just a risk for candidiasis

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Systemic candidiasis in humans usually originates from strains that have long-term colonized mucosal surfaces – especially the gut – prior to the onset of infection. Likewise, mucosal candidiasis is caused by strains that colonize the oral or vaginal cavity, often for years without causing problems. While research has long focused on the pathogenesis of candidiasis, recent studies began to explore the consequences of colonization beyond infection risk.

Traditionally, *Candida*-naïve mice were used for infection experiments, whereas natural infections occur subsequent to colonization. It is, however, becoming increasingly evident that *Candida albicans* colonization profoundly influences the host's immune system, including responses to subsequent candidiasis. Therefore, results obtained in models using *Candida*-naïve mice might over- or underestimate the effects of immunomodulatory interventions on systemic candidiasis. In the first part of my talk, I will provide an overview of colonization-induced effects, the underlying mechanisms, and the open questions that remain. I will present unpublished data that indicate strain-to-strain variation in the magnitude of colonization-induced immune responses, with consequences for the susceptibility to systemic candidiasis.

The second part of my talk will address consequences of antibiotic treatment that affect establishment and course of fungal infections. There is mounting evidence that besides their intended antibacterial effect, antibiotics can influence host cells. We observed that pre-treatment with antibiotics significantly increased susceptibility of specific-pathogen free mice to systemic candidiasis. This effect was reproducible across SPF mice that differed in their microbiome composition prior to treatment. However, antibiotics had no effect on the susceptibility of germ-free mice, suggesting that changes in the microbiota, rather than a direct impact on host cell physiology, are responsible for the negative impact of antibiotics on host resistance.

Immunophenotyping revealed a profound impact of antibiotic treatment on immune cell numbers in different compartments, cytokine production after infection, and the antifungal efficacy of innate immune cells. The latter is likely to explain the significantly higher renal fungal burden observed in antibiotic-treated mice after infection. Intestinal colonization with *C. albicans* prior to infection, resembling the situation in patients, abrogates antibiotic-induced increased susceptibility. However, antibiotic treatment of colonized mice leads to stronger pro-inflammatory cytokine responses that persists for a prolonged period of time even after the fungus has been cleared. This might have clinical consequences, such as increased immunopathology, in patients that are not able to clear the infection. These examples highlight the complexity of interactions between bacteria, *Candida*, and the host, and the different ways in which these interactions can affect patients.

PP-1

Eight-year simulated drought to infer the impact of climate change on ectomycorrhizal and soil microbial communities in alpine spruce and larch forest

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Climate change is expected to increase drought frequencies and intensities in the near future, posing significant challenges to (sub-)alpine ecosystems and mountain forests. In this study, we conducted an eight-year rain-out experiment to assess the effects of prolonged drought on ectomycorrhizal and soil microbial communities in an alpine mixed forest in Austria, comprising spruces, larches, and swiss stone pines. Our analysis focused on ectomycorrhizal (ECM) communities, utilizing ECM morphotyping and Sanger sequencing for molecular identification. Additionally, we employed community-based metagenomics to study soil fungal and bacterial communities comprehensively. We expect to find a shift in ECM colonization patterns, with a higher presence of drought-tolerant species in the rain-out plots. Notably, ECM species exclusive to control plots would exhibit higher susceptibility to drought stress. The insights gained from studying ECM, fungal, and bacterial communities will be correlated with growth and stress response parameters of trees at the study sites, enabling a better understanding of how soil microbial communities influence the resilience of alpine trees to drought stress.

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PP-2

In vitro Susceptibility Testing of Rare Moulds "RaMo Study" An ECMM-EC/EFISG/ECMM/ISHAM initiative

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Introduction

In recent years, there has been a rise in fungal infections attributed to rare fungi, posing a notable clinical challenge due to their inherent resistance and diminished efficacy of conventional therapies. There is limited data available on their susceptibility profile which adds to the challenges, resulting in the absence of epidemiological cutoff values (ECOFF) and clinical breakpoints.

Objectives

In an ongoing international study, a substantial number of molecularly identified rare moulds (n=450) are undergoing evaluation for their susceptibility to various conventional and novel antifungals. Invasive moulds not displaying *Aspergillus, Mucorales, Scedosporium* and *Fusarium* species were enrolled.

Materials & Methods

The susceptibility patterns are analyzed by Etest and broth microdilution methods using Clinical and Laboratory Standards Institute (CLSI) as well as European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. A freely selected ECOFF of >1 mg/L was set for further analyses.

Results

We determined the susceptibilities of 150 rare moulds to amphotericin B with MICs of 0.03 to >16 by broth microdilution and 0.004 to >32 mg/L by Etest. About 40% of the isolates are potentially non-wild type against amphotericin B in broth microdilution and as well as in Etest.

Conclusion

As mentioned, the increasing challenge posed by rare moulds underscores the importance of gathering extensive MIC data. The knowledge of innate resistance in rare fungi is of high importance for treatment.

PP -3

An Automated *ilastik*-based Workflow for Fungal Cell Morphology Analysis, Counting and Data Analysis: Enhancing Throughput and Accuracy with *caactus* (cell analysis and counting Tool using *ilastik* software)

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Introduction: Traditional methods for quantifying different (fungal) cell morphologies in liquid media via microscopy are labor-intensive and subject to user-dependent variability. This study addresses these limitations by introducing a streamlined workflow for fungal cell analysis, leveraging *ilastik* software and Python scripting.

Aim of the Study: The aim is to provide a user-friendly method for counting and analyzing fungal cell morphologies, accessible to those with minimal programming experience. *caactus* integrates with *ilastik*, simplifying image segmentation, data preparation, and statistical modeling.

Materials and Methods: Fungal spores were cultivated in a 96-well plate, with images captured at various time points using a Zeiss Axio Observer microscope. For identifying cells in the images, segmentation and object classification was realized by chaining three *ilastik* workflows: Pixel Classification for semantic segmentation of cell boundaries, instance segmentation based on the boundary images in Multicut, and Object Classification. Python scripting enabled image pre- and post-processing, data analysis, and statistical modeling, complementing the *ilastik* workflow. Manual counting of printed images served as a control for accuracy assessment. Time from image acquisition to receiving count data was recorded for both manual counting and the *ilastik*-based workflow.

Results: The workflow significantly reduced time from image acquisition to determining cell development stages and enabled rapid statistical analysis compared to manual counting. It also minimized variability compared to manual counting across users.

Conclusion: *caactus* provides a high-throughput solution for automated cell analysis, integrating *ilastik* software with image processing and statistical modeling. This approach promises to accelerate research and enhance data consistency in biological studies.

PP-4

Sequence-guided targeted isolation of *Mortierellacae* from glacier forefields

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The usage of amplicon sequence variants (ASV) for the analysis of fungal and bacterial taxonomy is now a well-established method promising a high taxonomical resolution based on biological variations at a single nucleotide level. In the study by Mandolini et. al (2024), the soil of four glacier forefields (Dachstein, Griessen, Marmolada, Tsanfleuron) was investigated which included the extraction of fungal genomic DNA and the analysis of the fungal marker gene ITS2 as sequence amplicons. A subsequent taxonomic assignment revealed that some ASVs within the phylum *Mortierellomycota* could not be assigned to any species or genus. Based on the knowledge of which ASVs are present in a particular soil sample, this study aims to investigate, if a targeted isolation of cultures containing those expected sequence variants is possible. The focus lies on the sequence-guided isolation, description, and preservation of unknown and threatened members of the *Mortierellaceae* family. For characterization and phylogenetic analysis, both classical examinations of morphological criteria and DNA-based methodologies are applied, respectively. By expanding the knowledge of *Mortierellaceae* from glacier forefields, this study could offer a small insight into alpine glaciers as a habitat; an environment that is highly impacted and threatened by climate change.

PP-5

Occurrence of environmental Mucorales with clinical relevance in Tyrol

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Introduction

Fungi belonging to the *Mucorales* can cause severe Mucormycosis in patients with immunosuppression or other primary conditions like diabetes or trauma. Generally, Mucorales are found in diverse ecological niches like soil, decomposing material, water, air and dust from indoor environments. Still, whether the occurrence and growth of clinically relevant species in these habitats forms a major exposure and infection risk is not clear.

Aim of this work

This work investigates the species distribution of *Mucorales* in agriculturally utilized soils, air samples and indoor dust in Tyrol as potential sources of infectious agents and aims to correlate species occurrence to clinical prevalence and unusually high rates of *Lichtheimia* spp. among Mucorales infections.

Material and Methods

Soil samples from agricultural land sites in Tirol were collected in different seasons (spring, summer, autumn) and examined with culture-based techniques. Furthermore, dust and air samples were included. Species ID was done by PCR and Sanger Sequencing of the ITS1 region and obtained sequences were blasted against the ISHAM ITS database.

Results

As expected, *Mucorales* were found in all soils independent of the season of sampling. *Mucor circinelloides* was the most frequently isolated species and *Mucor spp.* in general were more abundant than other *Mucorales*. The diversity of *Mucorales* at the sampling sites differed to great extent. In general, the species richness was greatest in autumn, showing a seasonal pattern with an increase throughout the growing season. Dust and air samples are currently evaluated for species ID.

Conclusion

Mucorales are omnipresent in tyrolean agricultural soils but the species distribution depends on the type of soil, the sampling site, the season and the type of crop planted. Against our expectation, *Lichtheimia spp.* were not found so far. This, and other limitations of culture based identification method lead us to continue further investigations utilizing qPCR methods to fully detect the mucoralean diversity in our samples.

PP-6

Unveiling Hidden Treasures: Revising taxonomy of South American phlegmacioid Cortinarii

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Abstract

The genus *Cortinarius* (Pers.) Gray *sensu lato*, boasting over 3,000 species, stands as one of the most diverse groups of ectomycorrhizal mushrooms worldwide. Recent phylogenomic advancements have sparked a reclassification into several new genera, yet many Southern Hemisphere taxa remain enigmatically unplaced. Our study shines a spotlight on the stirp *Xiphidipus*, focusing on species flourishing in South American *Nothofagaceae* forests. These mushrooms are not only ecologically significant but also prized as gourmet edibles for their exceptional nutritional value. Type material is compared with recent collections from Chile and Argentina, using morphological and multigene phylogenetic analyses. Our findings reveal that *Thaxterogaster austroturmalis* forms a robust clade, uniting it with several varieties as well as *Thaxterogaster xiphidipu*s and *Cortinarius pugionipes* var. *azonatus*. Conversely, *Thaxterogaster austroturmalis* var. *macrosporus* emerges as a distinct species, alongside *Cortinarius longicaudus* and *Thaxterogaster myxoclaricolor*. This research underscores the critical importance of integrating morphological and DNA based taxonomic analyses for accurate species identification and highlights the need for re-evaluation of current taxonomic classifications. Our study contributes to a more comprehensive understanding of Cortinarius biodiversity, uncovering the hidden gems of South American mycology.

PP-7

Improvement in diagnosis of urinary tract infections caused by *Candida* spp. in patients with indwelling urinary catheters

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Background: Urinary tract infections (UTI) with *Candida* spp. are known as common nosocomial fungal infections worldwide. The usage of colony forming units (CFU) counting for diagnosis of *Candida* UTIs is discussed controversially in literature. Furthermore, clinical observation of aggregates of human and yeast cells in urinary tract catheters (UTC) is novel and could lead to inconsistencies in CFU counting and thus to a false diagnosis of *Candida* UTIs.
Material and Methods: Here we investigate the phenomenon of *Candida* aggregates in patients with UTC. We collect samples from patients where aggregates are clinically observed and investigate them using microscopy, CFU counting, and antifungal susceptibility testing. Further, we look into the reproducibility of *Candida* CFU counting in urinary samples.
Results: 24 samples with the clinical observation of aggregates have been collected so far. Aggregates showed different composition, with 42% (10/24) consisting of human and *Candida* cells. Within the aggregates containing *Candida* spp., species were distributed differently. To this point, 32 samples have been collected for the CFU investigation. So far, no statistical difference in CFU counting can be found within patients, nevertheless, differences of 102 CFU/mL for several samples can be observed.
Conclusion: We conclude, that the aggregates seen in UTCs can consist of *Candida* spp., but cannot be used as diagnostic marker for *Candida* UTIs. The CFU counting for *Candida* spp. currently implies, that it is no sufficient measurement for diagnosing *Candida* UTIs because of fluctuations for several patients. More samples are needed for a more precise investigation.

PP-8

Potential effects of climate change on brown algae-parasite interactions

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The effects of climate change are becoming obvious in a wide variety of ecosystems. One example is the increasing overall temperatures of the water in the world oceans. In this work, two perspective scenarios of temperature increase in the world ocean were used to test the response of a pathosystem involving the brown alga *Macrocystis pyrifera* and two oomycetes *Anisolpidium ectocarpii* and *Eurychasma dicksonii* (strains Eury05 and Eury96) and one phytomyxid (*Maullinia ectocarpii*) infecting it. We used complementary molecular (qPCR) and microscopy (bright field and fluorescent microscopy) methods to assess the influence of temperature on the growth and infection phenotype of the pathosystem at 10 °C, 15 °C and 20 °C. The preliminary results show that different parasites have different temperature optima, in particular the infection potential of *E. dicksonii* and *M. ectocarpii* decreases with rising temperatures and disappear at 20 °C, whilst *A. ectocarpii* seem to be favoured at higher temperatures. According to these observations, the effects of changing temperatures are likely to be mediated in a species-specific way in the interactions between parasites and hosts, potentially affecting the coastal habitat.

PP-9

Distribution of *Aspergillus terreus* in soil in Tyrol, Austria

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Aspergillus terreus, is a filamentous fungus known for its role as an environmental decomposer and an opportunistic pathogen. Although infections are uncommon, certain areas, such as Innsbruck, have a higher prevalence. In Tyrol, infections and natural occurrence seemingly are more common in the eastern (lowland) regions than in the western (upland) regions, suggesting a pattern. The objective of this study was to confirm the higher prevalence of *A. terreus* in lowland soils compared to upland soils, identify contributing factors, and verify soil as its reservoir.

Approximately 40 lowland and upland sites were selected for soil sampling, tested for the presence of *A. terreus* using plate culture methods. Soil properties like pH, moisture, organic matter, total carbon, and nitrogen were analyzed, along with site parameters such as altitude and weather conditions. The soil immersion tube method was used to assess the soil as a reservoir for *A. terreus*.

A. terreus was found to be more abundant in Tyrolean lowland soils (18%) than in upland soils (8%) based on analysis of 300 soil samples. Lowland soil samples had higher moisture content (28%) than upland samples (26%) and a lower pH (6.4) compared to upland soil samples (6.6). Furthermore, upland samples contained more organic matter (9.0%) than lowland samples (7.2%).

Although differences were found in the soils of the Tyrolean upland and lowland, further research is needed to determine whether these factors also affect the distribution of *A. terreus* throughout Tyrol. One way to do this is through a planned genotyping study.

PP-10

Elucidating the contribution of AtrR in *Aspergillus fumigatus* triazole resistance

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Question

Clinically used azole antifungals inhibit a key enzyme in ergosterol biosynthesis, sterol 14-α demethylase (Cyp51), which leads to the accumulation of toxic sterol intermediates and depletion of ergosterol, eventually growth inhibition. In *Aspergillus fumigatus* one of the major transcription factors in sterol regulation is AtrR. In this work we aimed to investigate the effects of differently mutated AtrR on *A. fumigatus* azole resistance.

Methods

To allow transcriptional fine-tuning and study expression level-based effects of different *atrR* variants, tunable promoters were used. Azole susceptibility of different mutant strains was analyzed phenotypically by radial growth assays. Minimum inhibitory concentrations (MICs) were detected using the EUCAST-based broth microdilution method. Expression levels of major AtrR target genes such as *cyp51A* encoding the azole drug target and *cdr1B* coding for an azole efflux pump, were measured in AtrR mutants employing Northern analysis. Simultaneous detection of the subcellular localization of non-mutated and mutated AtrR was achieved by tagging genes with fluorescent proteins.

Results

We uncovered AtrR variants that exert a negative impact on azole resistance. Overexpression of N-terminally truncated AtrR in a wildtype, results in downregulation of *cyp51A* and *cdr1B* and significantly higher azole susceptibility. Notably, the same overexpression does not interfere with growth in the absence of azoles. Microscopic examination of N-terminally truncated AtrR revealed that its subcellular localization is only partially aligned with the wildtype version.

Conclusions

The AtrR N-terminus is essential for correct cellular localization of AtrR and its regulatory action. Expression of N-terminally truncated AtrR perturbs the regulatory function of wildtype AtrR, which results in defective expression of resistance-associated target genes and, as a consequence, increased azole susceptibility.

PP-11

A polyketide synthase gene cluster in *Trichoderma atroviride* is involved in 6-pentyl-alpha-pyrone biosynthesis

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The filamentous fungus *Trichoderma atroviride* is commonly used as a biocontrol agent against crop diseases caused by phytopathogenic fungi due to its high antagonistic activity that includes mycoparasitism and the production of antifungal and antibiotic secondary metabolites (SM). One of these bioactive and mycoparasitism associated SMs is 6-pentyl-α-pyrone (6-PP). We previously showed that 6-PP production in *T. atroviride* reaches its maximum when the fungus is cultivated in darkness or reduced light conditions but is largely inhibited by white light. Despite being one of the most significant SMs produced by *T. atroviride*, the molecular pathway underlying 6-PP biosynthesis has not yet been elucidated.

In this study, we demonstrate that 6-PP is biosynthesized via the polyketide biosynthesis pathway, and identified the PKS core gene essential for its biosynthesis in *T. atroviride*. Using CRISPR/Cas9, we conducted a successful *pks* gene knockout experiment and confirmed the role of this gene in 6-PP biosynthesis via subsequent HPTLC and LC-MS analyses. Deletion of the *pks* gene did not lead to major alterations in mycelial growth or pigmentation but resulted in a significantly reduced inhibitory activity against the plant pathogens *Botrytis cinerea* and *Rhizoctonia solani*. Additionally, we characterized the PKS gene cluster associated with 6-PP biosynthesis at the transcriptomic level, providing valuable insights into the molecular mechanisms underlying this biosynthetic pathway in *T. atroviride*. Finally, a similar pattern of production of 6-PP and 2-pentylfuran was observed which could indicate similarities in the biosynthetic origin and/or physiological role of these two substances.

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PP-12

The acyltransferase SidF is involved in biosynthesis of fusarinine-type and ferrichrome-type siderophores in *A. fumigatus*

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The opportunistic human pathogen *Aspergillus fumigatus* employs two high-affinity uptake mechanisms for iron: reductive iron assimilation (RIA) and siderophore mediated iron acquisition (SIA), being SIA crucial for its virulence in diverse infection models. *A. fumigatus* produces fusarinine-type (triacetylfusarinine C (TAFC)) and ferrichrome-type siderophores (ferricrocin (FC)). The first committed enzymatic step for all siderophores is hydroxylation of ornithine catalyzed by SidA. Subsequently, the pathways for synthesis of fusarinine- and ferrichrome-type siderophores split. For fusarinine-type siderophores an anhydromevalonyl group is linked to hydroxyornithine mediated by the transacylase SidF, while for ferrichrome-type siderophores an acetyl group is linked by the transacylase SidL and a yet unknown enzyme. Both SidF and SidL belong to the GNAT (Gcn5-related N-acetyltransferases) protein family but showing similarity only in the C-terminal half. SidF is localized in peroxisomes and the encoding gene is induced by iron starvation, while SidL is a cytosolic enzyme and expression of the encoding gene is largely iron-independent.

Here we found that simultaneous inactivation of both SidF and SidL abrogates biosynthesis of both fusarinine- and ferrichrome-type siderophores. Our studies also revealed an interdependence of fusarinine- and ferrichrome-type siderophores as inactivation of SidF blocked biosynthesis of TAFC but increased production of FC. Moreover, we demonstrate that truncation of either the GNAT-motif containing C-terminal or the N-terminal half blocks all SidF functions.

Taken together, this study suggests that SidF is the so far unknown enzyme catalyzing acetylation of hydroxyornithine, accepting acetyl-CoA and anhydromevalonyl-CoA as substrates for acylation of hydroxyornithine for biosynthesis of both fusarinine- and ferrichrome-type siderophores.

PP-13

The binding capacities of three filamentous fungi to remediate floating microplastic particles

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Plastic pollution, an inevitable and inadvertent anthropogenic thread, is known to have significant negative impacts on all global ecosystems exerting significant negative impacts on food webs. While animal, plant and microbial metabolic products are generally thought to be degradable by microorganisms, for anthropogenic chemicals like plastic polymers full *in situ* degradation is long-lasting, estimated to take place within hundreds to thousands of years. On the contrary, passive removal, e.g. through surface adhesion on living biota, can remediate pollutants within hours to days rather than years. In this study, three filamentous fungi – *Aspergillus niger*, *Aspergillus terreus* and *Penicillium rubens* – were used to investigate their potential to remediate polyamide particles of 5 µm size from a liquid environment. Removal rates of 59 to 67% could be proven by thermogravimetric analysis applying a fungal incubation time of 24 to 72 hours. Microscopic examinations allowed to visualize that the particles were tightly attached to fungal hyphae, consequently overgrown by succeeding layers of mycelium to restrain particles within the fungal pellet. These findings reveal great potential for future mycelia-based applications such as regenerative and sustainable “biofilters” eg. to clear flowing water.

PP-14

Pre-treatment with *Trichoderma viride* – towards a better understanding of its consequences for anaerobic digestion

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Understanding biological pre-treatment strategies for an enhanced production of bio-methane (CH_4) via anaerobic digestion is a central aspect in second generation biofuel research. In this regard, the application of fungi as a pre-treatment agent seems highly promising, however, understanding the mode of action is crucial. Here, an investigation applying an aerobic pre-treatment strategy for the decomposition of crystalline cellulose with the celluloytic fungus *Trichoderma viride* is presented. It could be shown that the pre-treatment affects substrate degradability during subsequent mesophilic, anaerobic digestion although no significant impact, neither positive nor negative, on the overall methane yield was found during batch fermentation. Short chain organic acids accumulated during anaerobic digestion resulting in changed methane production rates of treated and untreated substrate. Via Gompertz modelling changed overall degradation dynamics including methane production kinetics were evidenced. Finally, 16S rRNA amplicon sequencing revealed differences in microbial community dynamics, thus, relative abundances of key species were significantly affected by fungal pre-treatment depending on the duration of the pre-treatment.

PP-15

Are *Penicillia spp.* and *Talaromyces spp*. large scale producers of potent anticancer PDT-agents?

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Cancer remains a highly threatening illness that requires new therapeutic approaches. One promising strategy is photodynamic therapy (PDT). This therapy is highly selective because it employs photosensitizers that only become toxic when activated by light. Therefore it is ensured that cells remaining in the dark are unaffected. Some known photosensitizers belong to the chemical group of anthraquinones, which are colorful pigments found in plants such as certain species of the genus *Hypericum* [1], as well as in other natural sources like *Penicillium* spp. [2]. Due to their easy cultivation, especially *Penicillium* and *Talaromyces* species have garnered interest as cell factories for producing natural pigments on a commercial scale [3].

The central aim of this project is to identify potent fungal photosensitizers that selectively kill cancer cells and can be feasibly cultivated on a large scale. The discovered substances will be tested for their photocytotoxic activity against cancer cell lines from the nasopharynx and bladder, as these tissues can be illuminated minimally invasively. Amongst other cell lines, testing will be conducted using the FaDu (hypopharynx carcinoma), TE-1 (esophageal carcinoma), and RPMI 2650 (nasal septum carcinoma) cell line. In order to screen for suitable metabolites, we will use the OSMAC approach (One Strain MAny Compounds)[4]. It postulates that a single strain can produce various metabolites when subjected to different cultivation conditions. To trigger the production of potentially photoactive anthraquinones, we selected about 20 different species of *Penicillium* and *Talaromyces* and cultivated them on nine different media, in two light conditions (dark and light), and at two different temperatures (25°C, 30°C).

Here we present the preliminary results of our screening. In cell culture testing, a clear cytotoxic effect was primarily observed with *Talaromyces islandicus CBS 117284*, beginning from concentrations as low as 0,625 µg/mL. Light toxicity on the tested cancer cells was significantly enhanced by 15 minutes of irradiation with blue light (λ = 478 nm). Putatively responsible metabolites were annotated by the means of HPLC-DAD-MS (high-performance liquid chromatography coupled with diode-array detection and mass spectrometry).

The Austrian science fund (FWF) is kindly acknowledged for financial support (FWF P37163).

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PP-16

Spot on! Light-activatable pigments of South American *Cortinarii*

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The genus *Cortinarius* represents one of the most species-rich groups in the fungal kingdom. A chemotaxonomic characteristic of this genus is the presence of light-activatable pigments, specifically anthraquinones [1]. Given the increasing development of bacterial resistance mechanisms against common antibiotic classes, the use of natural compounds with light-activated antimicrobial effects presents a promising alternative treatment approach [2]. This so-called photodynamic therapy (PDT) is a medical treatment that utilizes photosensitizing agents activated by specific wavelength of light to generate reactive oxygen species, which induce cytotoxic effects on targeted cells, including cancer cells and pathogens [3].

This study investigates the light-induced activatability of anthraquinones found in *Cortinarius* species as potential photosensitizers. Since South American *Cortinarius* species are poorly researched compared to their European counterparts, they represent a substantial reservoir of undiscovered bioactive compounds.

To achieve this, we examine the relative singlet oxygen yields and the antimicrobial activity of these anthraquinones against ubiquitous human pathogens such as *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*. Light-induced toxicity against carcinoma lung (A549) and bladder (T24) cells is investigated, as well as pigment patterns are analyzed using High-Performance Liquid Chromatography (HPLC) and Thin-Layer Chromatography (TLC).

Here we present a comprehensive overview of the clade-specific screening conducted to date, revealing bioactivities that correlate with the observed pigment patterns.

The Austrian science Fond (FWF), Project POSADEC ZFI058670, is kindly acknowledged for their financial support.

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PP-17

Tracing different fractions of spiked environmental DNA using multiplex dPCR assays

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The direct extraction and analysis of the total environmental DNA (eDNA) enables the routine study of microbial communities at large scale and high taxonomic resolution. However, eDNA consists of both extracellular (exDNA) and intracellular DNA (iDNA), revealing even more profound insights into the diversity of currently prevalent communities and/or past assemblages. Since the 1980s, a multitude of methodological approaches for the separation of exDNA and iDNA from environmental samples has been published. Even though many of them are conceptually similar, methodological biases are poorly evaluated due to the lack of appropriate experimental set-ups. Here, we used two strains of both *Escherichia coli* and *Bacillus subtilis* to simultaneously trace and distinguish exDNA and iDNA of each selected model organism within spiked environmental matrices. Due to differences in the genomic location of specific marker genes, unique primer sets were developed for each strain, allowing their absolute quantification using multiplex dPCR assays. This methodological approach was successfully applied to different environmental matrices including soil, sediment, compost and sludge from anaerobic digestion and could therefore be extended to include also eukaryotic microorganisms. Hence, it highlights the potential to better understand the methodological ambiguities related to the separation of exDNA and iDNA, and thus to facilitate the validation and further optimization of such protocols.

PP-18

Immunophilins Conserved Across Three Phytomyxid Pathogens And Associated Production Of Reactive Oxygen Species

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Abstract

Immunophilins are a family of proteins observed in a broad range of organisms, including bacteria, fungi, plants, and animals. Immunophilins span three subfamilies, such as cyclophilins, FK-506 binding proteins, and parvulins. They are essential for various functions, including protein folding, fungal virulence, and in oxidative stress response. Phytomyxids, which comprise Phagomyxida and Plasmodiophorida, constitute the obligate biotrophic pathogens of oomycetes, diatoms, brown algae, and plants. However, there is not much data on the presence of immunophilins in phytomyxids. Here, we examined the immunophilins in the transcriptome of two phytomyxid pathogens parasitic of distinct hosts, such as *Maullinia ectocarpii* and *Spongospora subterranea,* and analysed the protein structure and domains. We also investigated the generation of reactive oxygen species (ROS) in *M. ectocarpii.* We observed the conservation of immunophilins across these phytopathogens, and also the protein domains. There was the light-independent generation of superoxides ($O_2^{-\bullet}$) across various stages of infection. Furthermore, the identification of the principal site of ROS generation, mitochondria in a similar pattern as that of $O_2^{-\bullet}$ in the infected cells, strengthened this assumption. The immunophilins could have also contributed to the increased production of ROS, and we anticipate that these findings may provide insights into the defence reaction in these pathogen-host interactions.

PP-19

Identifying cell wall related processes in brown algae during infection with *Maullinia ectocarpii*

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Maullinia ectocarpii is an obligate biotrophic parasite infecting brown algae, including *Ectocarpus siliculosus* Ec32. Infection results in hypertrophic infected host cells and likely leads to modifications in the cell wall of the host. Little is known about the cellular processes affected by the infection. Here, we investigate processes related to cell wall synthesis and modifications using a RNA-seq dataset of *E. siliculosus* Ec32 infected with *M. ectocarpii.* We additionally immunolocalise brown algal cell wall components during *M. ectocarpii* infection with specific antibodies. Key enzymes involved in the cell wall synthesis (including alginate and fucan synthesis) were differentially expressed in *E. siliculosus* Ec32 during *M. ectocarpii* infection. Cell wall modifying enzymes were differentially expressed as well and tended to be upregulated during infection. This leads to the conclusion that infection with *M. ectocarpii* leads to modifications in the cell wall of its host.

PP-20

Phenotyping of *Talaromyces wortmannii* and *Beauveria brongniartii* regarding morphology, growth and chemical diversity by combination of OSMAC – approach and co-culturing

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The regnum of fungi is described as a logical and relatively unexploited source of biotechnologically and pharmaceutically relevant compounds [1,2]. Analysis of fungal genome sequences have shown that fungi poses a more complex secondary metabolism than previously assumed. So-called silent gene clusters contain the information for the biosynthesis of a broad spectrum of secondary metabolites [3]. The expression of these clusters strongly depends on the growth conditions (e.g. growth medium, temperature, pH). This phenomenon is now being utilized by the so called OSMAC – approach (one strain many compounds) [4]. In order to trigger the biosynthesis of bioactive metabolites the method of co-cultivating multiple organisms in the same environment is gaining more attention within the scientific community [5,6].

The overall aim of this study was obtaining information on suitable growth conditions that favour the biosynthesis of biotechnologically relevant secondary metabolites. In this context, the combination of OSMAC approach and co-cultivation should provide a fundamental understanding of the phenotypic plasticity of *Talaromyces wortmannii* (CBS 235.38) and *Beauveria brongniartii* (BIPESCO 2). Within this context, this study provides a phenotypic characterization of *T. wortmannii* and *B. brongniartii* on solid media with focus on the influence of the growth medium and the co-cultivation of both organisms on the morphology, growth and chemical diversity of the cultures. To achieve this aim the study includes the evaluation of macroscopic morphological characteristics and surface area growth as well as the elucidation of the secondary metabolite profile by UHPLC-HRMS/MS analysis and data organization employing feature based molecular networking.

In accordance with observations in earlier studies the results show a clear plasticity in the phenotype of both organisms, with differences in the morphological characteristics of the colonies depending on the culture medium. Differences in the morphology of the colonies regarding vitality and pigmentation in co-culture compared to monocultures were also observed. Although there was no general tendency of increased chemical diversity in the co-cultures compared to the monocultures, up to 21% of observed features could only be detected in the co-cultures. Furthermore, co-cultivation led to an increased synthesis of certain secondary metabolites by the fungi. By employing feature based molecular networking the annotation of MS-spectra to known bioactive secondary metabolites of both organisms such as Talaromanin A, Flavomannin A, B and C, Oosporein, Enniatin A as well as Bassianolide was possible.

In conclusion the combination of OSMAC – approach and co-cultivation proved to be an effective method for screening the secondary metabolome of filamentous fungi while also providing a technique

to better understand the factors at play for the biosynthesis of bioactive secondary metabolites. At the same time feature based molecular networking provided a powerful tool for the interpretation of metabolomic data.

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PP-21

Taxonomy and host range of Polymyxa graminis (Phytomyxea)

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Polymyxa graminis is a soil born obligate biotrophic pathogen which belongs to the Rhizaria. Its main host are Poaceae (sweet grasses), although it does not cause any above ground symptoms to the host it acts as a vector for plant viruses such as the mosaic wheat virus. Despite, that these viruses have a significant impact on the cereal production the taxonomy and biodiversity of *P. graminis* is still unresolved due to the lack of molecular studies. The aim of this study was to expand and analyse the biodiversity of *P. graminis* across the alpine region. 141 samples from different altitudes, soil compounds and Poaceae were collected. A database of thirty-eight with *P. graminis* infected Poaceae was established based on the rDNA (18S-ITS1-5.8-ITS2-partially 28S).

Based on published sequences from NCBI and our dataset we established a Phytomyxea tree with a higher resolution of the biodiversity of *P. graminis* in the the whole phytomyxea clade. Furthermore, we used the combination of 18S and ITS rRNA for the inner diversity where we would like to establish at least one or two new f. sp. (forma specialis) to the existing five.

PP-22

Intratumoral microorganisms and their effect on antitumor immunity

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The discovery of intratumoral microbiota has led to research uncovering their diversity and their impact on anticancer immunity. These discoveries can be used for diagnosis and for therapeutic strategies such as immunotherapy in cancer. The impact of intratumoral microorganisms on antitumor immunity are highly species-specific and many relationships still need to be elucidated. To unravel some of these mechanisms we assessed intratumoral fungi, bacteria, and viruses in ovarian serous cystadenocarcinoma (OV) and pancreatic adenocarcinoma (PAAD).

We identified intratumoral species in the raw RNA sequencing data of The Cancer Genome Atlas for both cancer types and assessed their diversity within and between samples, revealing that microbial diversity is more dependent on tissue of origin than tumor occurrence. The abundance of different species correlated with several immune cell types and diverse immune pathways. In addition, we identified microbial peptides, which could trigger an anti-tumoral immune response by similarity to tumor associated antigens, termed molecular mimicry. Several microorganisms in OV could be associated with estimated immune cell fractions, including tumor promoting M2 macrophages. The impact of microbial abundance on patient survival was investigated, and two species were found to have an impact on overall survival in OV and in PAAD, respectively. By overlapping all analyses, the most interesting species were the fungus *Verticillium dahliae* in OV and the bacterium *Actinomyces succiniciruminis* in PAAD, which could play an important role in the respective cancer.

In summary, this work has provided a basis for how intratumoral bacteria, fungi and viruses can be assessed based on RNA sequencing data, and has highlighted potential links to antitumor immunity.

PP-23

azg1 as negative selectable marker for *Trichoderma atroviride*

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Trichoderma atroviride is a mycoparasitic filamentous fungus that lives and proliferates in the soil, interacting with plants, other fungi and bacteria. It produces substances that protect the plants from pathogenic fungi, while cell-wall degrading enzymes and secondary metabolites are released to fulfill its mycoparasitic activity against phytopathogens. Due to the large use of pesticides in agriculture, environmental isolates of *Trichoderma* are often exhibiting resistance to almost all common antifungal used in laboratories. This resistance presents a significant challenge in the characterization and genetic manipulation of *T. atroviride*, with the hygromycin resistance cassette being the only available marker to date.

The *azgA* gene encodes a membrane transporter for adenine, hypoxanthine, guanine and the toxic base-analog 8-azaguanine. It was previously shown in *Aspergillus nidulans* that deletion of *azgA* was conferring resistance to the nucleobase analog 8-azaguanine. To explore the potential of using this transporter as a selectable marker in *T. atroviride*, we identified and deleted the ortholog of the *azgA* gene, named *azg1*, using CRISPR/Cas9 technology. An initial characterization of the *azg1* deletion strain was performed on various media and under several stress conditions to assess any related phenotypes. The deletion strain showed no phenotypic differences from the wild type under all tested conditions, except for its resistance to 8-azaguanine. These findings indicate that *azg1* can serve as an effective negative selection marker for *T. atroviride*, facilitating future genetic studies in this species.

PP-24

Antifungal activity of secondary metabolites of the endophytic fungus, *Paraleptosphaeria sp*., against *Botrytis cinerea*

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Botrytis cinerea is one of the most common agricultural pathogens which imposes significant economic loss of between 10 to 100 billion dollars annually. This necrotrophic pathogen can infect over 200 plant species such as wine trees, causing grey mould, evident as grey fluffy mycelium. Recent studies indicate that *B. cinerea* can develop resistance against conventionally used fungicides. Therefore, there is an increased demand for discovery of new potential fungicides to combat *B. cinerea.* An alternative approach for discovery of new antifungal agents against this emerging pathogen is utilizing the natural products derived from fungal endophytes. These symbiotic microbes are underexplored sources of bioactive substances and therefore hold a great potential for discovery of novel antifungals. In this study, we aimed for isolation and identification of the bioactive metabolites of *Paraleptosphaeria* sp., an endophytic fungus isolated from the leaves of *Picea abies*, and assessment of their antifungal activities against *B. cinerea*. The fungus was upscaled on a rice medium, then extracted using ethyl acetate. Subsequently, the extract was systematically separated by various chromatography techniques using silicagel, Sephadex LH-20, and C18, which ultimately yielded seven compounds. The structures of the compounds isolated were identified by 1&2-D NMR spectroscopy methods and LC-ESI-HRMS, resulting in three new (**1**-**3**) and three known (**4**-**6**) chromophilones, and a new versiol-like terpenoid (**7**). The absolute configuration of the compounds was established by comparison of experimental and calculated circular dichroism spectra. Ultimately, pure compounds were evaluated for their bioactivities against *B. cinerea,* all of which illustrated moderate to promising bioactivities. According to the results obtained, *Paraleptosphaeria* sp. can be considered as a promising source of unexplored secondary metabolites with potential antifungal activities.

PP-25

Optimizing CRISPR-assisted transformation in *Trichoderma* species using the negative selectable marker *azg1*

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Trichoderma is a genus of filamentous fungi renowned for their adaptability to diverse environmental settings and their broad-spectrum mycoparasitic activity against phytopathogens. Some *Trichoderma* species have been evaluated as non - chemical fungicides in the agriculture and are currently recognized as relevant biocontrol agents. Other species of the genus, instead, hold significant industrial relevance in biofuel production, textile, food and paper industries, as well as in bioremediation processes. The increasing importance of these species necessitates an adequate toolbox to characterize, study and improve selected strains and their performance. However, the limited availability of selectable marker for genetic manipulation, primarily restricted to hygromycin B, along with the challenges in efficient transformation and achieving homologous recombination, hampers the full exploitation of *Trichoderma'*s potential.

The advent of CRISPR/Cas9 technology has brought notable advancements in manipulating *T. atroviride*. Nonetheless, a robust and efficient protocol for *Trichoderma* transformation has yet to be established. In this project, we aimed to optimize CRISPR-assisted transformation in four *Trichoderma* species: *T. atroviride, T. reesei, T. asperellum* and *T. virens*. Utilizing a newly established negative selectable marker in *T. atroviride*, the *azg1* gene, we investigated its potential as an insertion site in the other tested species. Our findings suggest that *azg1* could serve as a versatile locus for genetic modifications across different *Trichoderma* species, facilitating more efficient and targeted genetic manipulations. Moreover, close analysis of different steps of the transformation process allowed us to identify specific key moments that are highly relevant to the final success of the whole transformation.

PP-26

Life without peroxisomes? Deletion of *pex3* in *Trichoderma atroviride.*

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Trichoderma atroviride is a widely recognised mycoparasite used to protect plants from harmful fungal diseases. The role of peroxisomes in this lifestyle and the contribution of peroxisomes to the production of secondary metabolites is poorly understood. We deleted the *pex3* (peroxin3) gene, which encodes a protein that plays a critical role in the de novo formation of peroxisomal membranes from the endoplasmic reticulum and the maintenance of peroxisomes. We used a combined approach of ribonucleoprotein (RNP)-mediated CRISPR/Cas9 delivery and a transiently stable telomere vector, allowing marker-free gene deletion and vector recycling. *Δpex3* deletion mutants were confirmed by locus-specific genotyping PCR and sequencing. *Δpex3* strains exhibited biotin auxotrophy and reduced conidiation, consistent with other fungal peroxisomal biogenesis mutants. Furthermore, the ability to parasitise *Rhizoctonia solani* was reduced in *Δpex3* compared to wild type. Whether these mutants actually lack peroxisomes remains to be tested by localization studies with GFP-labelled peroxisomal proteins, and differences in secondary metabolite production have not yet been tested. However, we have shown, that the combined deletion approach produces the correct desired deletion strains in few transformants to be screened without the tedious generation of knock-out cassettes for classical gene replacement. Our results also suggest that peroxisome function contributes to mycoparasitism in *Trichoderma atroviride*.

PP-27

The BolA Family Protein Bol3 is Dual Localised by Alternative Translation Initiation in *A. fumigatus*

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Numerous enzymes involved in various metabolic pathways depend on iron-sulphur clusters (FeS) as co-factors. The biosynthesis of FeS requires complex biosynthetic machineries: the mitochondrial core FeS assembly machinery generates [2Fe-2S] clusters, representing precursors of mitochondrial and cytosolic [4Fe-4S] clusters. Trafficking of [2Fe-2S] has been shown to involve BolA family proteins in cooperation with glutaredoxins (Grx). In the mold *Aspergillus fumigatus*, mitochondrial [2Fe-2S] biosynthesis and the cytosolic/nuclear glutaredoxin GrxD have been shown to be essential for iron sensing. Most eukaryotes possess genes encoding BolA homologs with and without mitochondrial targeting sequences (MTS). In contrast, both *A. fumigatus* homologs possess putative MTS, which suggests the lack of cytosolic/nuclear versions. However, closer inspection of the Bol3 protein sequence revealed a methionine residue, located downstream of the MTS and highly conserved in various *Aspergillus* species. Proteomic analyses identified a Bol3 peptide that indicates that this methionine is derived from alternative translational initiation. Generation and phenotyping of different *bol3* mutant strains that lack the Bol3-encoding gene (*Δbol3*), the putative cytosolic/nuclear Bol3 (*bol3M41L*) or the mitochondrial Bol3 (*bol3M1L, bol3Δ38*) revealed different phenotypes, supporting a dual-localisation of Bol3. The most pronounced phenotype – a growth defect under iron limitation – was caused by the loss of the cytosolic Bol3. Fluorescence microscopy confirmed dual localisation of Venus-tagged Bol3 protein versions in mitochondria and the cytosol/nucleus. Purification of C-terminally Venus-tagged Bol3 proteins followed by nLC-MS/MS analysis revealed peptides, confirming different Bol3 proteins derived from alternative translational initiation, followed by proteolytic processing. Interestingly, this analysis indicated that mitochondria and the cytosol/nucleus contain the very same Bol3 protein discriminated only by N-terminal acetylation of the cytosolic/nuclear form. Mutation of the initial Kozak sequence demonstrated that increased translation initiation at the first AUG decreases translational initiation at the downstream AUG.

PP-28

Elucidation of species-specific fungal siderophore recognition

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Iron is an essential cofactor for several cellular processes. Despite its high abundance in the earth crust, the bioavailability of this metal is very low due to oxidation by atmospheric oxygen. Consequently, fungi evolved high-affinity iron uptake mechanisms including siderophore-mediated iron acquisition. Ascomycota and Basidiomycota produce exclusively hydroxamate-type siderophores that are subclassified into fusarinines, coprogens, ferrichromes, and rhodotorulic acid, whereby siderophore-class production is highly species-specific. Additionally, most fungal species are able to utilize xenosiderophores, i.e, not self-produced siderophore classes. For example, the mold *Aspergillus fumigatus* is able to utilize ferrioxamines, which are bacterial hydroxamate-class siderophores.
Recent studies elucidated the substrate specificities of four siderophore-iron transporters (SITs) of *A. fumigatus*, which, as shown here, facilitates prediction of the substrate specificity of fungal orthologs. Phylogenetic analysis of SITs from diverse representatives of Ascomycota, Basidiomycota and Mucoromycota revealed insights into the evolutionary conservation of SITs as well as the diversity of siderophore utilization by different species. For example, in contrast to *A. fumigatus*, *Aspergillus nidulans* is shown to be able to utilize the bacterial catecholate-type siderophore enterobactin via the SIT MirA supported by the genomic clustered enterobactin-hydrolysing enzyme EstA. Moreover, the so far uncharacterized SIT SitC was found to improve coprogen uptake in coprogen-producers such as *Aspergillus terreus* in comparison to the non-coprogen producer *A. fumigatus*. As siderophores might improve or decrease iron availability of other niche inhabitants dependending on their siderophore utilization spectrum, the production of a particular siderophore acts as either a cooperative or a competitive trait. Therefore, the elucidated diversity in siderophore-class utilization most likely reflects ecological niche adaptation, mirroring differences in cooperative and competitive interactions.

PP-29

Biosynthesis of silver nanoparticles using *Penicillium ochrochloron* and *Saccharomyces cerevisiae* extracts

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Silver nanoparticles (AgNPs) are used in many areas of daily life due to their specific size- and shape-dependent antimicrobial, optical and electrical properties [1, 2]. The bio-based production of high-quality and biocompatible nanocomponents represents an environmentally friendly and resource-saving alternative to conventional physicochemical manufacturing [3]. Fungi possess a broad spectrum of enzymes, proteins, cofactors and other metabolites that can serve as important reducing and stabilizing agents, making them promising candidates for the production of NPs.

In this study, AgNPs were generated using extracts of *Saccharomyces cerevisiae* and two different *Penicillium ochrochloron* strains (CBS 123 823 and CBS 123 824) by applying optimization strategies during the cultivation and biosynthesis, respectively. The NP production efficiencies were explored in dependence of environmental factors such as cultivation temperatures or the presence or absence of light. The resulting nanomaterials were characterized by spectrophotometric analysis, scanning electron microscopy, and energy dispersive X-ray spectroscopy.

Our first findings demonstrate that adaptations in the cultivation as well as in the biosynthesis phase but also organism specific traits can considerably affect the AgNP yield as well as the particle size: For example, light-mediated AgNPs synthesis based on *S. cerevisiae* resulted in more than 90 % increased yields and a reduced particle dimension from 130 nm to 100 nm in contrast to dark reaction conditions. Extracts of both *P. ochrochloron* strains resulted in similar high AgNP yields, but strain dependent differences in the AgNP size. The bio-based NP synthesis is a highly sensitive process where key parameters can be identified to modify unique particle properties.

Funding

This work was funded by the Standortagentur Tirol within the K-Regio Project SUPREMEbyNANO (P7440-016-036 to LC)

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