SYMPOSIUM

# Molecular Biology of Fungi 2026

Technische Universität Braunschweig

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BioSense Solutions

SARSTEDT

# Keynote speaker



## A travelling-wave strategy for plant–fungal trade

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For nearly 450 million years, mycorrhizal fungi have constructed networks to collect and trade nutrient resources with plant roots. Owing to their dependence on host-derived carbon, these fungi face conflicting trade-offs in building networks that balance construction costs against geographical coverage and long-distance resource transport to and from roots. How they navigate these design challenges is unclear. Here, to monitor the construction of living trade networks, we built a custom-designed robot for high-throughput time-lapse imaging that could track over 500,000 fungal nodes simultaneously. We then measured around 100,000 cytoplasmic flow trajectories inside the networks. We found that mycorrhizal fungi build networks as self-regulating travelling waves—pulses of growing tips pull an expanding wave of nutrient-absorbing mycelium, the density of which is self-regulated by fusion. This design offers a solution to conflicting trade demands because relatively small carbon investments fuel fungal range expansions beyond nutrient-depletion zones, fostering exploration for plant partners and nutrients. Over time, networks maintained highly constant transport efficiencies back to roots, while simultaneously adding loops that shorten paths to potential new trade partners. Fungi further enhance transport flux by both widening hyphal tubes and driving faster flows along 'trunk routes' of the network. Our findings provide evidence that symbiotic fungi control network-level structure and flows to meet trade demands, and illuminate the design principles of a symbiotic supply-chain network shaped by millions of years of natural selection.

# Molecular biology of environmental opportunism in *Trichoderma*

Irina Druzhinina

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*Trichoderma* (*Hypocreales*, *Ascomycota*) is among the most intensively studied genera of filamentous fungi, yet much of what is considered *Trichoderma* biology is inferred from a small number of experimentally tractable, application-relevant species. In the *Tricho*COSM whole-genus genomics project, we applied a phenogenomic framework integrating comparative genomics with more than 140 phenotypic parameters across 37 phylogenetically representative strains. This analysis revealed an ancient, genetically cohesive but physiologically diverse genus in which mycoparasitism and adaptation to arboreal microbial environments are widespread, whereas terrestrial persistence, endophytism and other forms of environmental opportunism have arisen repeatedly through lineage-specific combinations of character displacement and ecophysiological convergence. Genotype–phenotype associations further implicate regulatory genes, stress-response functions and poorly characterized gene families as important components of this diversification.

The same ecological plasticity that underlies many beneficial applications of *Trichoderma* also raises biosecurity concerns, as some opportunistic lineages can become pathogens of plants, fungi and humans. I will discuss an evolutionary framework in which recurrent forest disturbance generates spatially and temporally heterogeneous ecological opportunities that favour opportunistic phenotypes. Consistent with this framework, wildfire can transiently restructure forest-floor fungal communities and promote proliferation of *Trichoderma* together with other environmental opportunists.

Finally, I will illustrate how ecological niche expansion can be linked to molecular mechanism. In the coastal species *T. asperelloides*, regulatory variation affecting the accessory glycerol-pathway gene *gld1* alters osmotic tolerance, competitive fitness and persistence under salinity, directly connecting gene regulation with expansion into saline habitats.

Together, these findings support environmental opportunism as a conditionally expressed composite phenotype in which genomic capacity is realized through regulatory state, environmental sensing and ecological context. Understanding this ecological genomics is therefore essential for predictive bioprospecting and evidence-based biosecurity assessment of *Trichoderma* strains intended for agricultural and biotechnological applications.

# Convert the lignocellulose into bulk chemicals through consolidated bioprocessing technology using filamentous fungi

CHAOGUANG TIAN

*Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences*

The production of fuels and chemicals from renewable plant biomass has been proposed as a feasible strategy for global sustainable development. However, the economic efficiency of biorefineries is low. In our lab through metabolic engineering *Myceliophthora thermophila*, a cellulolytic thermophilic fungus, was constructed into a platform that can efficiently convert lignocellulose into important bulk chemicals, including malic and ethanol, without the need for extra hydrolytic enzymes. The malic titers of > 200 g/L from crystalline cellulose and 150 g/L from plant biomass (corncob) were achieved during fed-batch fermentation. In terms of cellulosic ethanol, the constructed engineering strain can directly ferment cellulose into ethanol by mimicking the aerobic ethanol fermentation of yeast (the Crabtree effect), including optimizing the synthetic pathway, enhancing the glycolytic rate, inhibiting mitochondrial NADH shuttles, and knocking out ethanol consumption pathway. The final engineered strain produced ethanol over 50 g/L directly from corncob, without the need for any added cellulase, while the starting strain produced almost no ethanol. We also demonstrate that as the ethanol fermentation by engineered *M. thermophila* increases, the composition and expression of cellulases that facilitate the degradation of cellulose, especially cellobiohydrolases, changes. The simplified production process and significantly increased chemicals yield indicate that the fungal consolidated bioprocessing technology that we develop here (one-step, one-strain) is promising for fueling sustainable carbon-neutral biomanufacturing in the future.

## Patterns of inheritance in basidiomycete fungi

Hanna Johannesson

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In sexual organisms, inheritance of new mutations is highly dependent on the timing of germline definition. In my group, we used the fairy ring–forming fungus *Marasmius oreades* to challenge the general assumption of a late germline separation in the Fungi. We collected mushrooms from different parts of rings over a 7-year period and identified new mutations in different tissues by whole-genome sequencing. We found evidence that fertile and sterile tissues had accumulated different mutations, suggesting that the germ line, destined for spore production, is already defined in the mycelium in this species. Moreover, the germ line carried fewer mutations than sterile tissues, indicating a lower mutation rate. Our findings suggest that early germline sequestration is more widespread than previously considered across multicellular life. Nevertheless, when extending the study to include the major lineages of basidiomycetes, we found that the feature of an early germline sequestration have occurred relatively recent in the family *Marasmiaceae*, and is not widespread among fungi.

Genomics & Evolution: Keynote

## Towards a complete understanding of antifungal resistance

Christian Landry

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Only a handful of antifungal molecules are regularly used in clinics and agriculture; resistance to these molecules can evolve during treatment or even segregate among naturally occurring populations of opportunistic pathogens. In a context where new drugs are slowly being developed and drug targets are limited, it is imperative to develop approaches and strategies to understand resistance mechanisms, slow resistance evolution, and prevent the spread of mutations. We combine synthetic biology, high-throughput sequencing, and literature mining to fully document antifungal resistance mutations and quantify their fitness cost and cross-resistance. In this seminar, I will present our most recent work on this topic, discuss how it can be used for precision antimicrobial treatment, and explore how it could be used to develop new drugs to overcome the most frequent resistance mutations.

# Ectomycorrhizal fungi: lessons learned from comparative genomics and transcriptomics

Annegret Kohler

*INRAE Centre Grand Est-Nancy*

To elucidate the genetic basis and evolution of the ectomycorrhizal lifestyle, we took advantage of the large-scale fungal genome sequencing programs underway at the Joint Genome Institute.

Comparing genomes of mycorrhizal fungi, wood decayers, and litter decomposers revealed several independent transitions from saprotrophy to mutualism. A general trend across ectomycorrhizal genomes-though not seen in ericoid or orchid mycorrhizal genomes-is the reduction of genes encoding plant cell wall-degrading enzymes (PCWDE). Nevertheless, ectomycorrhizal species appear to have retained distinct PCWDE repertoires depending on their ecological niche.

The availability of mycorrhizal genomes has also allowed us to compare mycorrhizal transcriptomes and identify symbiosis-related genes. We showed that *Laccaria bicolor* relies on both conserved and clade-specific genes—including effector-like mycorrhiza-induced small secreted proteins (MiSSPs)—to establish symbiosis with the roots of its host tree, *Populus*. Notably, conserved mycorrhiza-induced genes showed little overlap across species, suggesting that independently evolved mycorrhizal lineages have co-opted different genes for symbiosis. Interestingly, in several cases, induced genes across lineages converge on the same functions—such as small secreted proteins, transporters, or carbohydrate-active enzymes—without being orthologous.

Tree roots host a diverse fungal community, ranging from endophytes to mycorrhizal partners. In temperate and boreal ecosystems, ectomycorrhizal fungi dominate these associations, though endophytic fungi also constitute a significant part of the root mycobiome. While most molecular studies have focused on interactions between a single host and a single fungal partner, little is known about the factors shaping relationships among multiple beneficial fungi coexisting on the same host. We used poplar as a model system to investigate its interactions with several ectomycorrhizal and endophytic fungi. Through comparative transcriptomics, we identified *Populus* and fungal genes that directly or indirectly influence the establishment of ectomycorrhizal symbiosis and endophytic colonization.

# Sensing, Signaling & Metabolism



# Cell wall damage induces antibacterial defense in saprophytic mushroom *Coprinopsis cinerea*

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Saprophytic mushroom *Coprinopsis cinerea* inhabits herbivore manure, where it competes against a variety of other microorganisms, especially bacteria, over limited resources. Bacterial competitors do not only consume shared nutrients (exploitative competition) but also secrete antifungal effectors (interference competition). *C. cinerea* also responds with interference competition, inducing genes that encode antibacterial products (Kombrink et al., 2019). Remarkably, bacteria and bacterial cell free supernatant elicit overlapping transcriptional responses in *C. cinerea*. This indicates that induction of antibacterial defense does not require direct contact between the fungus and the bacteria but is rather mediated by soluble signals.

For a long time, the nature of these signals remained elusive. Here, we show that antibacterial genes in *C. cinerea* are induced by molecules that cause cell wall damage and activate stress signalling pathways required to maintain cell wall integrity. These molecules include reducing agents, proteases, and cell wall degrading enzymes (CWDEs). Conversely, membrane- and DNA- targeting compounds do not induce antibacterial genes. Consistently with these observations, we also found that *C. cinerea* response to bacterial strains impaired in CWDE secretion is significantly attenuated. We are investigating this further by monitoring the induction of antibacterial defense genes in *C. cinerea* strains deleted for cell wall-related pathways.

In conclusion, we argue that *C. cinerea* uses cell wall damage as a proxy for the presence of bacterial competitors. Our findings imply that bacterial competition in this context is mostly of the interference kind and exerted through specific classes of antifungal effectors.

## PmaI-dependent pH gradients initiate inter- and intraspecies germling fusion in filamentous fungi

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Somatic cell fusion among spore germlings is a complex process in filamentous fungi that enables the formation of a syncytial unit and facilitates the exchange of genetic material apart from sexual reproduction. The fusion process can be divided into three main parts: (I) the formation of conidial anastomosis tubes (CATs), (II) bidirectional chemotropic growth of CATs towards each other (CAT homing), and (III) cell fusion at the CAT contact side. In the last decades, substantial knowledge about genes and proteins involved in the fusion process was gathered, however, the signals provoking the bidirectional growth of the fusion partners towards each other remain unknown. In this study, we investigated the molecular mechanisms underlying germling fusion in the maize pathogen *Colletotrichum graminicola*, the grey mold *Botrytis cinerea* and the saprophytic model organism *Neurospora crassa*. Based on secretomes harvested during the germling fusion process of fusion-competent oval and fusion-deficient falcate conidia of *C. graminicola*, we identified pH as a communication signal in all three fungi. We showed further that the plasma membrane localized ATPase PmaI is a probable source for pH gradient formation, linking germination and germling fusion. In *C. graminicola*, we identified the a-phermone encoded by Cgppg-2, its cognate receptor CgSte3, and the scaffold protein CgSo of the Cell Wall Integrity (CWI) Mitogen Activated Protein Kinase (MAPK) pathway as responsible for the perception of acidic pH and subsequent downstream signalling. Since in Cgste3 and Cgppg-2 deletion strains chemotropic sensing of acidic pH is abolished, we propose that autocrine signaling might be a prerequisite for pH sensing. From our data, we propose a new model for germling interactions, in which pH serves as an interaction initiation signal, resulting in the activation of the cell-dialogue. Our findings have implications for our understanding of fungal communication and pathogenicity and highlight the importance of pH as a signal molecule in germling fusion.

# The lipid raft marker flotillin FloA enables relocalization of the plasma membrane H+-ATPase PmaA as part of a protective response to calcium stress

Moemi Kawashima, Thomas Krüger, Maira Rosin, Sophie Tröger-Görler, Thorsten Heinekamp, Axel A. Brakhage

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Biological membranes are not uniform but contain specialized regions called lipid rafts. These regions help organize membrane proteins and signaling molecules, but the functions of many lipid raft-associated proteins are still not well understood. One example is flotillin, a conserved lipid raft marker that has been linked to endocytosis.
In this study, we investigated FloA, the only flotillin homolog in the model fungus *Aspergillus nidulans*. We found that floA expression increases specifically under calcium stress, a response that is also seen in *Aspergillus fumigatus*. However, the regulation of floA differs between the two species: in *A. fumigatus,* it depends on the calcium-responsive regulator CrzA, whereas in *A. nidulans*, it does not. Using BioID proximity labeling, we identified proteins associated with FloA, including several proteins involved in endocytosis and the plasma membrane H+-ATPase PmaA. Under calcium stress, PmaA is internalized from the plasma membrane. In cells lacking FloA, this internalization is blocked, which leads to intracellular calcium overload and cell death.

Together, our results show that FloA is required for PmaA internalization during calcium stress and helps protect fungal cells from calcium-induced damage. These findings support a role for flotillin in endocytosis and stress adaptation in fungi.

## Volatile organic compounds in the mushroom *Cyclocybe aegerita*

Martin Rühl, Nikolas Hoberg, Annika Wagner, Daniel Krüzselyi, Karen Harms

*JLU Gießen*

The edible Agaricomycete *Cyclocybe aegerita* is an excellent model for studying the formation and biological roles of fungal volatile organic compounds (VOCs). Its complete life cycle can be reproduced under laboratory conditions, enabling the investigation of volatile production throughout fruiting body development. Recent studies have revealed that the volatilome of *C. aegerita* is highly dynamic and closely linked to developmental stage, with characteristic shifts from C8 oxylipins dominating vegetative growth to sesquiterpenes becoming prominent during sporulation.

This presentation will provide an overview of the major classes of VOCs produced by *C. aegerita*, their biosynthetic origins, and the molecular mechanisms underlying their formation. Particular emphasis will be placed on the biosynthesis of C8 oxylipins, including the characteristic mushroom odorants oct-1-en-3-ol and octan-3-one. Stable-isotope labeling experiments suggest the existence of two distinct biosynthetic branches leading to these compounds, while transcriptome analyses have identified candidate lipoxygenases, dioxygenases, hydroperoxide lyases, alcohol dehydrogenases, and ene-reductases involved in their formation. In addition, the talk will highlight recent advances in elucidating terpene biosynthesis in *C. aegerita*.

# A Novel Role for Pheromone-Like Receptors in the Basidiomycete *Schizophyllum commune*

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G protein-coupled receptors (GPCRs) mediate diverse cellular responses in fungi, including signaling required for sexual reproduction. In basidiomycetes such as the wood-rot fungus *Schizophyllum commune*, sexual development and mating are controlled by multiallelic and independent mating loci that encode components for mate recognition and signaling, including homeodomain transcription factors, pheromones, and GPCRs. Canonical pheromone receptors in basidiomycetes are homologs of the yeast receptor Ste3 and function in non-self recognition. In addition, Agaricomycetes encode pheromone-like receptors whose functions remain largely unknown and are not sufficient to trigger mating-specific development. Here, we investigate the role of one such pheromone-like receptor in polarity establishment and lateral branch formation. Overexpression of this receptor caused defects in lateral branching, as shown by confocal microscopy. To investigate underlying mechanisms, we combined RNA-seq and RT-qPCR with structural and phylogenetic analyses to examine transcriptional changes and evolutionary relationships among pheromone-like receptors in Agaricomycetes. Our results reveal a previously unrecognized function for this receptor group, demonstrating that pheromone-like receptors in Agaricomycetes have roles beyond the recognition of pheromones.

## Exploring Bioactive Metabolites in Nematode-Trapping Fungi

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Nematode-trapping fungi (NTF) have evolved specialized trapping structures to capture and kill nematodes, making them valuable models for studying fungal predation and the ecological functions of fungal secondary metabolites. My research focuses on the nematode-trapping fungus *Arthrobotrys oligospora* and aims to identify bioactive metabolites involved in fungal-nematode interactions.

Genome mining using antiSMASH revealed a diverse repertoire of biosynthetic gene clusters (BGCs), including terpene, polyketide synthase (PKS), nonribosomal peptide synthetase (NRPS), hybrid and other cluster classes across 15 NTF species. Comparative transcriptomic analyses identified several candidate BGCs in *A. oligospora* that are differentially expressed following exposure to Caenorhabditis elegans, suggesting that their metabolites may contribute to predation-related processes. Functional characterization of selected candidates is currently being carried out through targeted deletion of biosynthetic genes. Initial phenotypic analyses indicate that certain mutants exhibit developmental and predation-related phenotypes. To identify metabolites associated with these clusters, wild-type and mutant strains are being cultivated for comparative metabolomic analyses.

In parallel, wild-type strains are cultivated under diverse growth conditions to stimulate secondary metabolite production. Metabolite extracts are subjected to antinematode bioassays and activity-guided purification to identify active metabolites associated with nematode predation.

This work advances our understanding of the molecular basis of fungal carnivory and provides a framework for linking biosynthetic gene clusters, secondary metabolites, and predation-related phenotypes in nematode-trapping fungi.

# One MAPK, Many Signals: Insights from the HOG Pathway in Magnaporthe oryzae

Milena Runge, Dr. Stefan Jacob, Elena Klein, Dr. Lukas Stelzl, Dennis Martin, Antonia Preuß, Jonas Paulus

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Living cells decode and respond to a multitude of environmental cues using a limited number of signaling pathways. To investigate how signal specificity is achieved, we study the High Osmolarity Glycerol (HOG) pathway in the rice blast fungus *Magnaporthe oryzae*, one of the most destructive plant pathogens worldwide. In M. oryzae, the HOG pathway integrates osmotic, oxidative and heat stress as well as fungicide action by activation of the MAP kinase MoHog1. Upon dual phosphorylation of its conserved TxY motif (T171and Y173), MoHog1 translocates to the nucleus and induces stress-specific gene expression. We hypothesize that information about the nature of a stress signal is encoded in the phosphorylation pattern and intracellular trafficking of MoHog1. Using nanoUPLC-MS/MS, we found stress-specific differences in phosphorylation intensity and dynamics at T171 and Y173, suggesting that distinct stimuli are reflected in unique MoHog1 phosphorylation signatures. Analysis of phosphosite mutants further underpinned that T171 plays a particularly important role in downstream signal transduction and transcriptional activation. To explore the contribution of nuclear transport to signaling specificity, we visualized the subcellular localization of GFP-tagged phosphomutants and showed that nuclear import depends on dual phosphorylation. In addition to that, we investigated the importin β family protein MoNmd5. In both *S. cerevisiae* and human, the Imp7 ortholog Nmd5 is considered a major import factor for Hog1/p38 MAPKs, which interestingly lack a consensus Nuclear Localization Sequence. Surprisingly, deletion of MoNMD5 in *M. oryzae* did not prevent MoHog1 nuclear accumulation and did also not impair HOG pathway-dependent gene regulation under salt stress. These findings challenge the current model of p38-type (MoHog1) transport in pathogenic fungi and further point to alternative routes of signal transport into the nucleus. Our results suggest that *M. oryzae* may achieve signaling specificity through an array of stimulus-dependent phosphorylation patterns and we hypothesize that it creates even more diversity by the orchestration of Hog1-transport elements.

# Characterization of a stress-controlled circadian clock in *Alternaria alternata*

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Anticipating environmental changes during the course of a day is advantageous for many organisms and depends on an endogenous circadian clock. Circadian clocks are conserved from cyanobacteria to human and rely at the molecular level on positive and negative transcription-translation feed-back loops (Larrondo, 2025). In fungi the circadian clock has been analyzed in great detail in *Neurospora crassa* and was shown to also work in few other fungi. However, in some fungi, the clock is conditional and strongly depends on nutritional cues, or crucial components of the *N. crassa* clock are absent in fungi like *Aspergillus nidulans*. Here we studied *Alternaria alternata*, which harbors the Frq protein FrqA and the blue-light photoreceptor LreA as central clock components from *N. crassa* (Schuhmacher et al., 2024). Using the clock-controlled frqA promoter we constructed a luciferase-based *A. alternata* strain and were able to induce rhythmic expression of luciferase with white- or blue light or with temperature cycles for entrainment. However, a free run of the clock under constant conditions was barely visible. In order to decipher possible links to other signaling pathways, we analyzed the HOG pathway (Igbalajobi et al., 2020) and found that in hogA mutants the circadian clock showed robust free-run cycles. This novel interrelationship can be explained by physical interaction of FrqA with the HOG pathway transcription factor AtfA. We present evidence that the circadian clock in *A. alternata* is conditional, probably dependent on dynamic interactions between FrqA and AtfA. Experiments to understand the dynamics of the protein interaction are underway. Furthermore, we show that FrqA - and hence probably the circadian clock - plays a role in fungal interactions likely through the control of secondary metabolite production.

Larrondo, Luis F. (2025) Circadian rhythms: pervasive, and often times evasive. Philos Trans R Soc Lond B Biol Sci 380.1918: 20230477; Schuhmacher, L., Heck, S., Pitz, M., Mathey, E., Lamparter, T., Blumhofer, A., Leister, K. & Fischer, R. (2024) The LOV-domain blue-light receptor LreA of the fungus *Alternaria alternata* binds FAD or FMN as chromophore and acts as light and temperature sensor. J. Biol. Chem., 300(5):107238; Igbalajobi, O.A., Gao, J. & Fischer, R. (2020) The HOG pathway plays different roles in conidia and hyphae during virulence of *Alternaria alternata*. Mol Plant Microbe Interact. 33(12): 1405-1410.

# Sustainable fungal dyes: heterologous reconstruction of *Hypoxylon azaphilone* pathways in *Aspergillus oryzae*

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The growing demand for sustainable alternatives in the textile and leather industries highlights the need for natural pigments that avoid the environmental and health hazards associated with synthetic dyes. Fungi of the ascomycete genus *Hypoxylon* are prolific producers of azaphilones, a structurally diverse class of polyketide-derived pigments with promising applications in these sectors. However, the complex and often developmental stage-dependent regulation of their production in wild-type strains hinders the characterization of the biosynthetic pathways. To overcome this limitation, this study will focus on the heterologous reconstruction of azaphilone biosynthetic pathways in *Aspergillus spp*. cell factories to achieve a more comprehensive understanding of their synthesis and regulation.

As an initial step, genomic analysis of azaphilone BGCs across several *Hypoxylon spp.* was performed, revealing a highly conserved structure split across two separate BGCs.

Guided by these findings, the core polyketide synthases and associated accessory genes from *Hypoxylon fragiforme* will be transferred into *Aspergillus spp.* Transformants and gene expression will be analysed to verify stable genomic integration and functional expression. Furthermore, the secondary metabolites produced by the engineered strains will be extracted, fractionated, and profiled by HPLC-MS. Analysis of these metabolic profiles will allow for the detection of key intermediate compounds, enabling a precise stepwise reconstruction of the azaphilone biosynthesis.

Ultimately, characterizing these metabolic steps provides a critical foundation for the targeted engineering of these pathways. This knowledge is expected to support the tailored production of high-value, eco-friendly pigments, offering a sustainable alternative to synthetic dyes.

# Phase-Separated Khd4 Condensates Link RNA Regulation to ER Homeostasis in *Ustilago maydis*

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Successful infection by fungal pathogens depends on precise gene expression programs. A crucial component of this regulation is mediated by RNA-binding proteins (RBPs). However, the spatiotemporal mechanisms driving this RNA control remain largely unexplored. In the model corn smut fungus *Ustilago maydis*, the RBP Khd4 – a multi KH domain protein – undergoes phase separation specifically in proximity to the endoplasmic reticulum (ER) to support pathogenic development in the host.

In this study, we investigated the function of Khd4 in maintaining ER organization and morphology. Microscopic analyses revealed that loss of Khd4 results in severe disruption of ER architecture, indicating a critical role in ER assembly and maintenance. While this severe ER phenotype suggests a direct structural or trafficking-related function, Khd4's ability to target mRNAs raises the possibility that it regulates gene expression at the post-transcriptional level to achieve this.

To dissect these potentially distinct functions, we are mapping functional protein domains through truncation studies – specifically examining their role in driving phase separation – and characterizing Khd4's interaction network. We hypothesize that Khd4 coordinates ER homeostasis either directly through membrane trafficking pathways, or indirectly by utilizing phase-separated condensates to regulate the localization, stability, or translation of mRNAs encoding ER organizing factors. These studies aim to establish the mechanistic relationship between phase separation, membrane trafficking, spatiotemporal mRNA regulation, and ER integrity, revealing whether Khd4 acts as a multifunctional hub linking these critical processes during fungal pathogenesis.

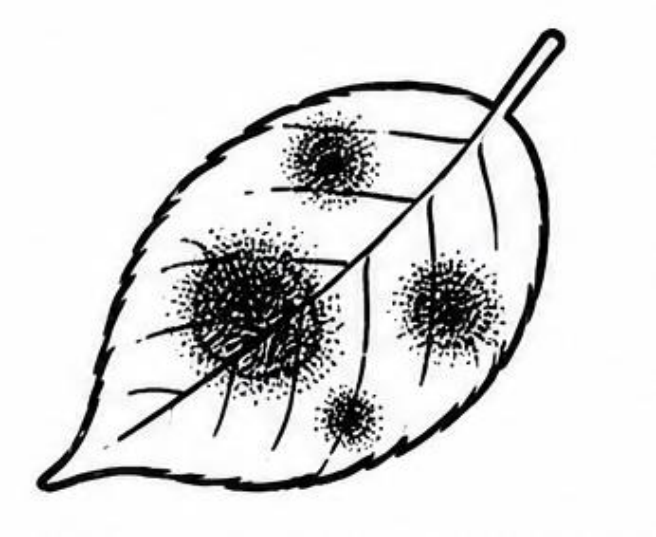
# Structural and functional dissection of EOP-1 reveals coupled disordered and folded regions governing $Ca^{2+}$-mediated spore germling interactions

Lina Strzelczyk, Peer Lukat, Ulrike Brandt and Andre Fleißner

*TU Braunschweig*

Cell-cell interactions are critical for fungal development, yet their molecular mechanisms remain elusive. In *Neurospora crassa*, genetically identical conidial germlings engage during colony establishment in a unique communication mode, alternating between sending and receiving signals, in order to interact and fuse with each other [Fleißner et al 2009]. Central to this dynamic exchange is the Early Oscillating Protein 1 (EOP-1), which is rhythmically recruited to the plasma membrane of interacting germlings and essential for cell fusion [Oostlander et al 2026]. This study aims to unravel the underlying molecular basis of the cell dialog mechanism by elucidating the molecular function of EOP-1. Bioinformatic predictions indicate an intrinsically disordered N-terminal region and a C-terminal AIM24-like domain [Abramson et al 2024]. Crystal structure determination of the EOP-1 AIM24-like domain suggested that the protein might assemble into homotrimers, which we confirmed by subsequent in vitro mass photometry measurements. Based on structure elucidation, we identified two $Ca^{2+}$-binding sites within the AIM-24-like domain. Using MST MonolithX to measure the affinity of EOP-1 to $Ca^{2+}$, we found that the AIM-24-like domain alone only binds $Ca^{2+}$ in a millimolar range, while the full-length protein showed an elevated affinity in a micromolar range. Moreover, functional complementation assays showed that neither domain alone rescues the eop-1 deletion, demonstrating that both regions are indispensable. Given that *N. crassa* germling fusion requires extracellular $Ca^{2+}$ [Chu 2013], we hypothesize that the binding of $Ca^{2+}$ by EOP-1 contributes to the subcellular dynamics and function of this signaling factor. Site-directed mutants of the predicted $Ca^{2+}$-coordinating residues in EOP-1 caused its mislocalization and abolished germling interaction, demonstrating that $Ca^{2+}$-binding is crucial for proper EOP-1 localization and function during early fungal development. Moreover, a cluster of cysteine residues resides at the trimer's core, suggesting that these thiols could serve as a redox sensor. Future studies should determine whether ROS-mediated redox modifications regulate EOP-1 function. Taken together, these findings demonstrate how distinct motifs and their functional roles influence germling interaction. This provides a basis for systematically analyzing the underlying molecular mechanism and reveals connections within the wider regulatory network.

# Fungal Interactions & Pathogenicity



# Facultative heterochromatin mediated by core and accessory chromosome-encoded H3K27-specific methyltransferases controls virulence in a fungal phytopathogen

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In many fungal phytopathogens, infection is regulated by accessory genomic regions enriched in facultative heterochromatin, but the precise role of this chromatin state in virulence is poorly understood. Here we examined the two facultative heterochromatic marks H3K27me and H3K36me in the tomato-pathogenic *Fusarium oxysporum* isolate Fol 4287, by genetically analyzing the corresponding histone methyltransferases Kmt6 and Ash1. Fol has three KMT6 homologs, two of which, KMTB/C, are encoded on an accessory chromosome. Loss of the core gene KMT6A resulted in strongly reduced but not abolished H3K27me, revealing that methylation of subtelomeres can be taken over by the accessory copy Kmt6b. To the best of our knowledge, this demonstrates for the first time that accessory fungal histone modifiers can contribute to the overall epigenetic profile. Deletion of KMT6A or ASH1 resulted in derepression of in planta-induced effector genes under axenic conditions. In the case of Ash1, H3K36-mediated H4 deacetylation is likely involved in gene silencing under non-inducing conditions. KMT6A deletion mutants were blocked at early stages of root infection and non-pathogenic on tomato plants, but were still able to kill the animal host Galleria mellonella. Constitutive overexpression of the pathogenicity-related transcription factor FTF1 in the Δkmt6a background restored effector gene activation in planta but not pathogenicity, suggesting that additional facultative heterochromatin-regulated virulence factors are essential for plant infection.

# Beyond effectors: hijacking host cytokinin signaling for rust pathogenesis in poplar

Chhana Ullah

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Plant pathogens overcome host immunity through effector-mediated suppression of defense signaling and enzymatic detoxification of antimicrobial compounds. However, the mechanisms by which pathogens manipulate host physiology to facilitate infection in long-lived trees remain poorly understood.

Here, we show that leaf infection in black poplar (Populus nigra) by the biotrophic rust fungus *Melampsora larici-populina* is associated with extensive reprogramming of cytokinin (CK) metabolism. While isoprenoid CKs, including trans-zeatin (tZ), accumulated rapidly after infection, poplar-specific aromatic CKs (topolins) remained higher throughout disease development. Exogenous application of tZ and topolins significantly enhanced rust colonization, demonstrating a direct role of CKs in promoting pathogen growth. Elevated CK levels reduced defense hormones, including salicylic acid and jasmonates, and suppressed antifungal flavan-3-ol accumulation. Moreover, CK treatment, particularly with poplar-specific ortho-topolin, increased free amino acid concentrations, potentially enhancing nutrient availability for the biotrophic pathogen.

Together, our findings reveal host cytokinin manipulation as a previously unrecognized pathogenicity mechanism in tree–pathogen interactions, enabling rust fungi to simultaneously weaken chemical defenses and enhance access to host nutrients to favor biotrophic growth.

# The secreted effector PefB from *Arthrobotrys flagrans* interferes with C. elegans alternative splicing

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Nematode-trapping fungi (NTF) are predatory fungi that are widely distributed in soil ecosystems. They capture nematodes by forming specialized trapping structures, including adhesive nets, sticky knobs, and constricting rings. Following capture, the fungi penetrate the nematode body and secrete a variety of hydrolytic enzymes and toxins that progressively degrade host tissues, ultimately leading to nematode death . Although hydrolytic enzymes are indispensable for fungal colonization, increasing evidence indicates that small-secreted proteins (SSPs), functioning as effectors, also represent important virulence factors during fungal infection. To date, three effector proteins have been characterized in *Arthrobotrys flagrans*. These effectors promote fungal infection by enhancing nematode paralysis or weakening the nematode cuticle.
In this study, we further functionally characterized the putative effector PefB. PefB is a 271-amino-acid long protein containing an N-terminal signal peptide and a predicted nuclear localization signal (NLS). Ectopic expression of PefB in *Caenorhabditis elegans* resulted in its accumulation within the host nucleus. PefB contains four serine/arginine (SR) repeat motifs and localizes to nuclear speckles after entering the host nucleus. Furthermore, PefB interacts with host SR proteins, thereby modulating host pre-mRNA splicing. Through interference with the host RNA splicing machinery, PefB alters alternative splicing and compromises nematode fitness, revealing a previously unrecognized virulence mechanism employed by nematode-trapping fungi. One of the target genes was the nematode transcription factor c-Jun1, a proto-oncogene, which along with c-Fos1 regulates the expression of many genes. The splice variant induced by PefB lacks BRLZ domain. This suggests a dominant-negative effect of c-Jun1. Expression of PefB or the PefB-induced splice variant of c-Jun1, both driven by a heat-shock promoter, cause severe body deformations. RNA-seq analysis of nematodes expressing PefB revealed a global increase in exon-skipping (SE) events. Functional enrichment analysis of differentially expressed genes further indicated that epithelial-associated biological processes were prominently affected, suggesting that PefB-mediated splicing perturbation extensively remodels epithelial gene regulatory networks. Our analysis points to a highly sophisticated fungal-nematode interaction rather than quick killing and digestion of the prey.

# The fungal transcription factor AbrR coordinates secondary metabolism and antibacterial defence in *Aspergillus fumigatus* during interspecies interactions

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*Aspergillus fumigatus* is ubiquitous in nature and is also an opportunistic human fungal pathogen that harbors more than 30 biosynthetic gene clusters (BGCs) for secondary metabolites, many of which remain transcriptionally silent under standard laboratory conditions. It has been shown that the otherwise silent fumicycline (fcc) BGC of *A. fumigatus* can be activated either by co-cultivation with *Streptomyces rapamycinicus* or by exposure to its secreted arginoketide azalomycin F. To elucidate the underlying regulatory mechanisms, we performed transcriptome analyses of *A. fumigatus* treated with azalomycin F and during co-cultivation with *S. rapamycinicus*. Both conditions elicited a coordinated antibacterial response, characterized by the induction of specific secondary metabolite pathways and antibacterial effector genes, accompanied by repression of other BGCs, including those responsible for fusarinine C, pyripyropene A, and fumagillin biosynthesis. Among the most strongly induced genes, we identified a zinc cluster transcription factor, designated AbrR (antibacterial response regulator), that is conserved across Ascomycota. AbrR expression was specifically triggered by azalomycin F, selected *Streptomyces* species, and soil-derived bacteria of the genus *Kribbella* and *Arthrobacter*. Functional analyses demonstrated that AbrR is essential for activation of the fumicycline BGC: its deletion significantly reduced, whereas its overexpression enhanced fumicycline production, even in the absence of external stimuli. Furthermore, AbrR operates upstream of the pathway-specific regulator FccR and controls multiple other antibacterial BGCs, including those involved in the biosynthesis of hexadehydroastechrome, helvolic acid, and xanthocillin, as well as multiple antibacterial peptides and proteins. The response pattern observed *in A. fumigatus* triggered by different soil bacteria reveal species-specific formation of antibacterial SMs and species-specific activity of fumicyclines highlighting their ecological function in interspecies competition. Together, these findings establish AbrR as a central regulator of *A. fumigatus* that coordinates the chemical defense in response to bacterial signals.

## *Diplodia sapinea* as a molecular model for forest pathology: dissecting the endophyte-to-pathogen transition

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Forests worldwide are increasingly threatened by climate change, which destabilizes tree-microbe interactions and can turn latent endophytes into aggressive pathogens. Yet forest pathology still lacks the tractable molecular model systems that have transformed our mechanistic understanding of crop diseases. *Diplodia sapinea*, a globally distributed fungal endophyte and pathogen of conifers, is a promising candidate to close this gap: it colonizes *Pinus sylvestris* asymptomatically but can switch abruptly to a pathogenic lifestyle, causing shoot dieback, cankers and tree death, particularly under drought and heat stress. To make this system experimentally accessible, we established a molecular and cell-biological toolbox for *D. sapinea*. This includes a reliable protocol for producing and storing highly viable pycnidiospores, spore-based infection assays on Scots pine seedlings at different developmental stages, and a space- and time-efficient assay that does not require greenhouse facilities. Wounded inoculations favor pronounced disease symptoms, whereas non-wounded plants show a higher percentage of asymptomatic infections, allowing endophytic and pathogenic states to be established under controlled conditions. We further developed an Agrobacterium-mediated transformation system, enabling targeted gene knockouts and the expression of fluorescent reporters for live-cell imaging of fungal growth in planta. Building on this platform, we are exploring the role of fungal secondary metabolite biosynthesis in the interaction with the host, using targeted knockouts to probe its contribution to the endophyte-to-pathogen transition. By opening up the molecular dissection of the endophyte-to-pathogen continuum, these tools and approaches aim to establish *D. sapinea* as a community model system for molecular forest pathology, offering a framework to understand and ultimately mitigate climate change-driven forest disease.

## Does adaptation help to survive in metal stress conditions?

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Disturbed or polluted areas strongly affect vegetation or re-forestation approaches. This might cause stress to the plants, particularly in combination with extreme weather conditions expected with climate change. The mutualistic symbiosis ectomycorrhiza formed by basidiomycetes with roots of shrubs and trees can help to stabilize forest health.

A strategy to obtain fungal isolates for inoculation, recultivation and remediation is to isolate new strains directly from disturbed or contaminated areas. Here, we tried something new and adapted a laboratory strain of the ectomycorrhizal fungus *Tricholoma vaccinum* to higher metal contents by transfer to medium containing increasing metal contents. Adapted strains were compared to wild type based on morphology, metal content and proteomics.

Upon cadmium treatment, higher biomass, a more compact growth, less reduced cell length and less Cd accumulation were considered as signs of lowering Cd stress through adaptation. The proteome of an adapted strain showed changes in, e.g., transporters, aldehyde dehydrogenases, or cytoskeleton components.

The performance of the adapted strains in the field will show if the adaptation can be used as a powerful tool to support reforestation in disturbed areas.

# Genetic dissection of the chemical defense of ink cap mushroom *Coprinopsis cinerea* against fungivorous nematodes

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Fungivorous nematodes are ecologically significant predators of fungal mycelia. The chemical defense of the model agaricomycete *Coprinopsis cinerea* against these predators includes both a constitutive (autonomous) and an inducible layer. The inducible layer is manifested by the production of a series of nematotoxic intracellular proteins in the vegetative mycelium of this fungus in response to grazing by the stylet-feeding fungivorous nematode *Aphelenchus avenae* (1). Interestingly, this defense layer is not restricted to the sites of grazing but propagates both acropetally and basipetally along trunk hyphae (2). The signals and signalling pathways triggering this defense response against nematodes and mediating its propagation in the mycelium of *C. cinerea* remain unclear. Moreover, the physiological significance of the constitutive and inducible chemical defense layers for the fungus has not been assessed so far.

We applied both forward (F) and reverse (R) genetics approaches to address some of the questions outlined above. (F): We found that there are large differences between various *C. cinerea* monokaryotic strains with regard to propagation of *A. avenae* on respective vegetative mycelia. To explore the genetic basis for these differences, a permissive (regarding nematode propagation) strain was crossed with a prohibitive strain and F1 generations and backcrosses thereof were produced. Phenotypic and bulk segregant analysis (BSA) of these progenies revealed a 400 kb genomic region whose SNPs highly correlated with the permissive phenotype. This region includes a previously characterized locus encoding three paralogous, highly nematotoxic proteins that are structurally and functionally related to bacterial and plant AB-toxins. (R): Transcriptomic profiling of *C. cinerea* in response to grazing by *A. avenae* revealed strong induction of several genes coding for protein kinases and transcription factors. Using an optimized CRISPR–Cas9 knockout method, more than 35 gene knockout mutants in these genes (and some nematotoxin genes) were generated to identify the signalling pathways responsible for triggering and propagation of the defense response against fungivorous nematodes in *C. cinerea* vegetative mycelium.

## The Sporisorium reilianum-specific effector protein SRS13906 targets a conserved signaling pathway to suppress plant immunity

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*Sporisorium reilianum f.sp. reilianum* (SRS) causes head smut disease in sorghum. The biotrophic fungus proliferates inside the plant until flowering stage without visible symptoms. A knock-out of the *S. reilianum*-specific effector gene SRS13906 results in visible plant defense responses including the induction of sorghum-specific phytoalexins and cell death on infected leaves, suggesting that the effector suppresses plant defense responses during early infection stages. We proved secretion of SRS13906 and showed that an N-terminal myristoylation site is targeting the effector to the plasma membrane. Fusing any kind of tag to the C-terminus results in non-functionality and instead induces a stronger phytoalexin response in colonized sorghum leaves. We found that SRS13906 can also suppress INF1-induced cell death, when transiently expressed in *Nicotiana benthamiana*. Fully functional tagged versions of the effector protein cannot be detected via Western blot neither when transiently expressed in *N. benthamiana* nor in infected sorghum. Taken together, it seems that the protein needs to be degraded for full function in sorghum. Fusing a C-terminal tag increases protein stability in *N. benthamiana* and sorghum. Preliminary co-immunoprecipitation mass spectrometry results hint at an interaction of the effector protein with LRR receptor complexes. This suggests that SRS13906 may function by affecting LRR receptor-complex turnover.

## Eisosomal proteins are essentialfor plant–fungal interaction of *Neurospora crassa* and the sweetgrass *Brachypodiumdistachyon*

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Plant fungal associations encompass a continuum from pathogenic to mutualistic interactions that have profoundly shaped terrestrial ecosystems. The ability of fungi to colonize living plant tissues typically depends on tightly coordinated signaling and membrane organizations processes. Recently, we described a previously unrecognized endophytic interaction between the filamentous model fungus *Neurospora crassa* and the sweetgrass *Brachypodium distachyon*. Contrary to its long-standing classification as a strictly saprotrophic species, *N. crassa* was observed to invade root epidermal and cortical cells and to colonize the vascular bundles without inducing disease symptoms, indicating astable non pathogenic association. To identify functional elements in this interaction, we systematically screened *N. crassa* mutants lacking key signaling and membrane-associated components. Disruption of certain signaling pathways including MAP kinase and NADPH oxidase mutants did not impair plant colonization. However, deletion of ncw-6 or div-23, two proteins associated with and localized to eisosomes, completely disrupted plant root colonization of *N. crassa*. Eisosomes are plasma membrane microdomains in fungi that contain transporters and integral membrane proteins. These findings suggest an integral function of eisosomes in plant–fungal interaction of *N. crassa* and possibly other fungi as well.

# A fungal effector hijacks a conserved GSK3 signaling hub to drive host gall formation

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Successful biotrophic pathogens remodel host tissues to generate an environment conducive to their own proliferation. How fungal effectors reprogram plant developmental pathways to achieve this remains largely unknown.

We show that the *Ustilago maydis* effector Iag1 promotes virulence by directly targeting the conserved GSK3-like kinase BIN2, a central negative regulator of brassinosteroid signaling. Through a conserved GSK3-interaction motif, Iag1 binds multiple maize GSK3 kinases, activates brassinosteroid signaling, and triggers extensive transcriptional and developmental reprogramming that culminates in gall formation. Loss of Iag1 severely compromises fungal virulence, demonstrating that manipulation of this signaling node is essential for disease development.

Unexpectedly, the interaction motif employed by Iag1 is also found in unrelated plant viral proteins and metazoan AXIN2-family regulators, revealing a striking case of convergent evolution toward a deeply conserved signaling interface. These findings identify plant GSK3 kinases as conserved vulnerability hubs repeatedly exploited by evolutionarily distant organisms.

Our work uncovers a molecular mechanism by which a fungal pathogen rewires host cell identity and establishes GSK3 signaling as a central target for pathogen-induced developmental reprogramming.

# Investigating the Presence of *Dothistroma spp.* and *Lecanosticta acicola* and co-infections with *Diplodia sapinea* and *Sydowia polyspora* in Various Urban Pine Tree Species in Germany with Comparison to Switzerland and France

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Urban trees face increasing vulnerability due to climate change and the ongoing spread of damaging fungal pathogens. Non-native tree species, often planted for aesthetic, biodiversity, or climate-adaptive value, are common in cities. However, these assemblages can act as pathogen hubs and sources of infection for surrounding forests. In Germany, a knowledge gap exists regarding the geographic and host ranges of fungal pathogens causing needle blight and shoot dieback on non-native pines in urban areas. This study uses systematic sampling and molecular methods to investigate the spread of *Dothistroma pini*, *Do. septosporum*, *Lecanosticta acicola*, and their co-infections with *Diplodia sapinea* and *Sydowia polyspora* across multiple European cities. A sampling campaign surveyed over 300 symptomatic trees across 12 cities in Germany, France, and Switzerland, encompassing 43 native and non-native pine tree species. Pathogen presence was detected using species-specific primer pairs: DPtef-F/DPtef-R (*Do. pini*), DStub2-F/DStub2-R (*Do. septosporum*), LAtef-F/LAtef-R (*Lecanosticta acicola*), DiSapi-F/Diplo-R (*Diplodia sapinea*), and SpolEF4F/SpolEF3R (*Sydowia polyspora*). Fungal cultures isolated from necrotic needle areas were also molecularly identified. Gel electrophoresis of PCR products confirmed 193 infections across a diverse range of hosts, including many co-infections. *Sydowia polyspora* and *D. sapinea* were the most common species, exhibiting high adaptability across diverse non-native hosts. Comparatively, the host and geographic ranges of *Dothistroma* spp. and *L. acicola* appeared more confined. Of the target pathogens, *D. sapinea* and *S. polyspora* were the first and second most common in *Pinus sylvestris*. Certain non-native hosts, e.g. *Pinus jeffreyi*, exhibited relatively high susceptibility. Specific regions and urban features (parks, cemeteries, and botanical gardens) were also identified as susceptibility hotspots. Pathogen detection in previously unrecorded areas provides molecular evidence of continued geographic spread, with several instances representing new host records. Notably, the common occurrence of co-infections suggests urban pines may face complex disease pressures. These insights can guide urban forest management regarding species selection and mitigation strategies.

**Keywords:** urban forestry, fungal pathogens, non-native *Pinus*, molecular monitoring, needle blight, co-infections

# Secondary metabolism as a driver of virulence and host adaptation in dermatophytes

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Dermatophytes are among the most successful fungal pathogens and are responsible for some of the most common human mycoses worldwide. Despite extensive research on classical virulence factors, the role of secondary metabolism in dermatophyte pathogenicity remains poorly understood.

In this study, we investigated members of the *Trichophyton benhamiae* species complex, a group of closely related dermatophytes that differ markedly in virulence. Comparative transcriptomic analyses performed under ex vivo infection-like conditions revealed pronounced differences in the expression of genes associated with secondary metabolism among individual taxa. In contrast, established virulence-related traits alone could not fully explain the observed variation in pathogenic potential.

These findings prompted us to examine secondary metabolite production in greater detail. GC-MS profiling revealed distinct volatile metabolite signatures across taxa, highlighting substantial diversification of secondary metabolism even within this closely related species complex. Several detected compounds exhibited antimicrobial activity, particularly against fungi, whereas others have previously been linked to immunomodulatory effects. Together, these observations suggest that secondary metabolites may contribute to adaptation to different host-associated niches and influence interactions between dermatophytes and their hosts.

Our results link variation in secondary metabolism with differences in virulence among closely related dermatophyte lineages and highlight secondary metabolites as a potentially important factor in dermatophyte adaptation and pathogenicity.

## Shared molecular logic of fungal interaction lifestyles: GPCR signaling from pathogenesis to symbiosis

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Fungi engage with other organisms across a wide spectrum of lifestyles, ranging from pathogenesis to highly integrated mutualistic symbioses such as lichens. In each case, successful interaction depends on the ability of the fungus to perceive its biotic environment and translate external cues into developmental, metabolic, and physiological responses. G-protein-coupled receptors are central regulators of fungal signaling and development, yet much of our mechanistic understanding of these processes comes from filamentous pathogens. However, it is becoming increasingly clear that mutualistic symbioses may rely on surprisingly similar molecular principles.

Here, I will discuss how structural classification can provide a route to identify and investigate fungal GPCRs beyond canonical pheromone receptors. Using the maize pathogen *Ustilago maydis* as one example, we identified a diverse repertoire of putative GPCRs, including receptors outside established fungal GPCR classes. One of these receptors, Gpe1, is essential for successful plant infection and is activated by a peptide derived from the genomically neighboring effector protein Pit2. This peptide is released after Pit2 is processed by plant papain-like cysteine proteases, thereby linking receptor activation to entry into the plant environment. We then applied this structure-guided logic more broadly and identified a larger group of basidiomycete GPCRs with similarity to rhodopsin-like receptors, although the Pit2-derived ligand appears to be absent outside closely related smut fungi. Beyond this specific receptor-ligand pair, structural analysis of Gpe1 revealed conserved microswitch-like features that provide a framework to identify related receptors and to experimentally modify their signal transduction capacity.

Extending this approach to lichen-forming fungi revealed expanded Pth11-like receptors not only in pathogens but also in symbiotic fungi, suggesting that pathogenic and mutualistic lifestyles may rely on overlapping sensory architectures. Interestingly, we also found related but distinct microswitch patterns in these GPCRs. Our findings further raise the possibility that fungal GPCRs may be less diverse but more structurally constrained than previously anticipated. Together, our work establishes new routes to discover, classify, and functionally characterize fungal GPCRs.

# Herbicidal Phenolic Compounds from the wood-decaying fungi, such as *Biscogniauxia nummularia*

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Fungi inhabiting the tissues of forest-forming trees in Central Europe pose an increasing threat due to climate change. Under abiotic conditions unfavorable to plants, such as drought, they are capable of shifting from an endophytic lifestyle to pathogenic. One such fungus is *Biscogniauxia nummularia*, which primarily targets European beech (*Fagus sylvatica*).

In our project, we aim to investigate the mechanisms by which this fungus operates. We are examining how and when the fungus attacks host tissues, as well as how it spreads within them. To achieve this, we are developing a GFP-labeled strain and conduct infection experiments with beech trees under various conditions to monitor its spreading over time using confocal laser scanning microscopy. At the same time, we are tracking the infection on gene expression level. Here we focus on genes of fungal enzymes specifically involved in the production of phytotoxic phenolic compounds to find out if they are involved in the pathogenicity mechanism of *B. nummularia*.

Furthermore, we are assessing the potential of these phenolic compounds as resource for bioinspired herbicide alternatives in agriculture in the consortium. For this, substances are chemically improved and tested against weeds. Our data demonstrated that alternative natural chemicals isolated from plant pathogenic fungi have the potential to find new solutions for weed management in conventional as well as organic agriculture.

# Does differential localization of effector orthologs contribute to host specificity of *Sporisorium reilianum*?

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*Sporisorium reilianum* is a host-specific biotrophic plant pathogen causing head smut disease on maize (*S. reilianum f. sp. zeae*, SRZ) or sorghum (*S. reilianum f. sp. reilianum*, SRS). Maize leaves colonized with SRZ show minimal cell death suggesting the presence of cell death-suppressing effectors. An INF1-induced cell death suppression screen revealed one functional effector candidate (sr16441). This effector carries a predicted chloroplast targeting peptide (cTP), and effector-GFP fusion proteins retain the ability to repress cell death and showed chloroplastic localization upon expression in *Nicotiana benthamiana*. In contrast, the orthologous effector of SRS (srs_16441) lacks a predicted cTP, localizes to the nucleus and the cytoplasm, and lacks cell-death suppression activity. SRZ strains lacking the effector sr16441 show reduced virulence. We will show the effect of effector deletion on proliferation of the fungus in maize. In addition, we will use the GFP-sr16441 fusion protein to identify interaction partners in *N. benthamiana* by Co-IP/MS analysis. In parallel, we are testing whether wrong effector localization of srs_16441 is the reason for non-functionality in INF1-induced cell death suppression in *N. benthamiana* by the generation of chimeric SRS/SRZ effector versions. In summary, the data hint at a localization-dependent function of sr16441 in the SRZ/maize pathosystem. It will be interesting to determine whether the non-chloroplastic effector srs_16441 contributes to virulence in the SRS/sorghum pathosystem.

# Deciphering the Molecular Mechanism of a Fungal Effector Protein that Impairs Plant Development

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The fungal effector protein SAD1 of the biotrophic fungus *Sporisorium reilianum* (SRZ) induces sub-apical ear formation in maize via loss of apical dominance. Expression of SAD1 in *Arabidopsis thaliana* also results in increased inflorescence branching. The E3 ubiquitin ligase ZmRGLG1 has been identified as an interaction partner by yeast two-hybrid screening. The ZmRGLG1 homolog of *A. thaliana*, AtRGLG2 and its paralog AtRGLG1, but not the related proteins AtRGLG3, AtRGLG4, or AtRGLG5, also interact with SAD1 in one-on-one yeast two-hybrid assays. In-planta interaction between SAD1 and ZmRGLG1/2 and AtRGLG1/2 was observed using BiFC. However, Co-IP of SAD1 with AtRGLG1/2 was not successful possibly indicating that interaction of SAD1 with these E3 ubiquitin ligases could be transient.

In search of additional interaction partners, the SAD1 effector protein was immunoprecipitated from SRZ-infected maize leaves and from stable *Arabidopsis thaliana* lines expressing SAD1. LC/MS analysis resulted in the identification of AtTPL (TOPLESS) and its maize homolog RELK2 as potential interaction partners. TOPLESS and its relatives function as co-repressors in various biological processes such as floral organ identity determination, phytohormone signalling and immune responses. To verify the importance of interaction of SAD1 with TOPLESS, we will do and present one-on-one yeast two-hybrid and Co-IP interaction studies, complemented by RT-qPCR analysis of TOPLESS-regulated transcripts from plants expressing or not SAD1. This may indicate that SAD1 induces subapical ear formation by interacting and possibly interfering with multiple plant proteins.

# Does alternative effector splicing contribute to host specificity of *Sporisorium reilianum*?

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The fungus *Sporisorium reilianum* exists in two formae speciales, causing head smut of sorghum (SRS) or of maize (SRZ). The effector gene 13906 contributes to virulence of SRS on sorghum, and effector deletion increases plant defence reactions, visible as red phytoalexin induction in colonized sorghum leaves. In *Nicotiana benthamiana*, the effector can suppress INF1-induced cell death and may interact with NbSERK3, an INF-1 receptor-associated protein. The ortholog in SRZ is transcribed into two splice variants, SP1, nearly identical to the variant in SRS, and SP2, that is translated into a shortened modified protein. We found that 13906 gene deletion in SRZ reduced virulence on maize and induced stunted growth. To know whether both splice variants of SRZ contribute to virulence on maize, we express each splice variant in the SRZ 13906 gene deletion strains. Results of the infection experiment will be presented.

When expressed in SRS, both splice variants of SRZ seem to have a function: While SP1 completely complements loss of 13906 in SRS, expression of SP2 leads to a partial complementation, sometimes showing phytoalexin formation at altered locations. We are currently evaluating whether both splice variants can (partially) suppress INF1-induced cell death in *N. benthamiana*. Next, we will test by bimolecular fluorescence complementation (BiFC), whether one or both splice variants of SRZ can interact with NbSERK3.

If both splice variants can interact as well to NbSERK3 as 13906 from SRS, this would hint that alternative 13906 effector gene splicing is not a major contributor to host specificity of *S. reilianum*.

# Comparative Effectoromics of Nematophagous Fungi Reveals Lineage-Specific Predatory Repertoires

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The fungi *Arthrobotrys oligospora* and *Pleurotus ostreatus* have evolved different strategies to capture and exploit nematodes. While chemical cues and toxins have been implicated in nematode attraction and killing, the role of secreted effector proteins in modulating nematode physiology remains largely unexplored. In this study, we applied an established secretome prediction pipeline integrating signal peptide prediction, transmembrane domain identification, and subcellular localization analysis to define the potential effectoromes of *A. oligospora and P. ostreatus*. Comparative analyses revealed that the biophysical properties of these effector-like proteins are broadly similar to those found in non-nematophagous fungi. Genomic organization analysis showed that many effector-like genes form genomic clusters, with those in *A. oligospora* displaying a strong enrichment in subtelomeric regions, genomic compartments often associated with accelerated evolution and adaptive diversification in fungi. Using comparative genomics and structure-guided analyses, we further identified species-specific effector repertoires uniquely present in *A. oligospora* or *P. ostreatus*. Notably, several genomically clustered effector-like genes are strongly induced during the formation of nematode-capturing structures, suggesting coordinated roles in fungal predation These genomically clustered and highly induced effector-like genes represent promising candidates for future loss-of-function screening to investigate their roles in fungal predator–prey interactions.

# Dual Localization of the GPCR GprC in *Arthrobotrys flagrans*: Mechanistic Insights into Mitochondrial Targeting via Alternative Translation

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Nematode-trapping fungi (NTF) are soil-dwelling filamentous fungi capable of transitioning from a saprotrophic to a predatory lifestyle upon starvation and prey sensing. In the NTF *Arthrobotrys flagrans*, the G-protein coupled receptor GprC senses ascarosides, which are nematode-derived pheromones, triggering the trap formation process. Interestingly, GprC exhibits a unique dual localization at the plasma membrane and at mitochondria, where it increases cellular respiration (1). However, the mechanisms underlying this dual targeting and its intramitochondrial role remained unknown. Utilizing bioinformatic predictions and fluorescence microscopy, we identified an alternative variant of GprC, designated GprCtail, which is comprised only of the last two transmembrane domains, putatively translated via an internal ribosome entry site (IRES) and selectively localizing to the mitochondria. Furthermore, bimolecular fluorescence complementation (BiFC) assays demonstrated a direct interaction between GprCtail and the mitochondrial import receptor Tom70, suggesting that GprCtail utilizes the carrier pathway for translocation and subsequent insertion into the inner mitochondrial membrane (IMM). To our knowledge this is the first example for an IRES in fungi. We also show that the same mechanism is used for mitochondrial localization of a GPCR of *Alternaria alternata* and *Aspergillus nidulans*. Experiments to identify the molecular targets of GprCtail at the electron transport chain of *A. flagrans* are under way. Together, our research provides critical insights into the predator-prey dynamics of NTFs and elucidates a novel mechanism for the dual localization and mitochondrial function of a fungal GPCR.

(1) Hu, X., D. Hoffmann, M. Wang, L. Schuhmacher, M.C. Stroe, B. Schreckenberger, M. Elstner, and R. Fischer. 2024. GprC of the nematode-trapping fungus *Arthrobotrys flagrans* activates mitochondria and reprograms fungal cells for nematode hunting. Nat Microbiol. 9:1752-1763.

## Exploring the Pathosystem *Ramularia collo-cygni* in Barley

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Ramularia leaf spot is a major disease of barley (Hordeum vulgare), causing substantial yield and grain quality losses. Biotic cause of the disease is the hemibiotrophic fungus *Ramularia collo-cygni*, which is transmitted via seeds and air. Following an initial asymptomatic colonization, the pathogen switches to a necrotrophic phase during the onset of leaf senescence. Disease management is challenged by the limited availability of resistant barley cultivars and the declining efficacy of major fungicide classes. Despite extensive research, key aspects of R. collo-cygni biology and host interaction remain poorly understood, largely because the pathogen is not yet an established experimental model.

To facilitate functional studies, we first established a robust protocol for conidiospore production, providing reliable inoculum for controlled infection studies. Based on this, an artificial infection system was developed to investigate disease development. To this end spores were also used to establish an Agrobacterium-mediated transformation protocol. For microscopic analyses of host–pathogen interactions, *R. collo-cygni* was transformed with eGFP and for viability testing with a GusPlus construct.

The established spore transformation system enables efficient site-directed homologous recombination for targeted gene deletion, achieving high homologous integration rates and providing a valuable platform for functional genetic studies in *R. collo-cygni*.

# Integrative taxonomic revision of the *Arthroderma quadrifidum* complex and *Arthroderma curreyi*: molecular, morphological, and ecological perspectives

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Dermatophytes are keratin-degrading fungi. Most *Arthroderma* species are taxonomically well defined, but the *Arthroderma quadrifidum* complex remains unclear. It includes morphologically similar isolates from diverse environments, such as cold cave sediments, hibernating bats to and clinical samples from animals and humans. To clarify the taxonomy and evolutionary history of this group, this study employs an integrative approach using a broad dataset that includes historical strains and recently described relatives. Because the species are morphologically cryptic, differentiation relies on multi-locus phylogenies reconstructed using ITS, tef1α, and β-tubulin markers. These molecular data are synthesized with comprehensive phenotypic analyses, including morphology and temperature dependent physiology, alongside metadata obtained from the GlobalFungi database. Furthermore, initial phylogenies will guide the selection of representative isolates for whole-genome sequencing. Ultimately, this genomic and phenotypic synthesis aims to uncover lineage divergence, mating strategies, and the evolutionary adaptations driving host colonization and environmental transitions within the complex.

# Biological control approaches of *Hymenscyphus fraxineus*, the causal agent of ash dieback

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The causal agent of ash dieback, *Hymenoscyphus fraxineus* [synonym: *H. pseudoalbidus*], is native to East Asia, but was accidentally introduced into Europe. The main symptoms on the European ash (*Fraxinus excelsior*) include shoot dieback, necrotic lesions, but also root collar necroses, which reduce tree vitality and timber quality, and cause tree mortality. Consequently, the pathogen, which threatens the survival of ash populations, is associated with severe economic and ecological damages outside its native range.

Innovative approaches for disease management are needed. Within the FraxForFuture project, the use of fungal endophytes, priming and mycoviruses were investigated. We assessed the efficacy of endophytes in vitro and under greenhouse conditions, where they reduced disease development associated with H. fraxineus. In vitro assessments of the fungal metabolites produced in antagonism assays elucidated structures of anti-fungal metabolites, providing insights into the interactions. We also investigated the hypovirulent effect of two mycoviruses in planta. Priming of saplings with a low virulent strain of *H. fraxineus* under greenhouse conditions showed that priming has particular promise for reducing disease severity. Further experiments are planned to evaluate the use of bacteria and yeast as antagonists, as well as to investigate the role of microbial synergies in further reducing symptom development.

Overall, these results contribute further insights into the complex interactions between a pathogen and other microorganisms in vitro and in planta, especially in relation to disease development. These insights can be used to facilitate the possible identification of biological control approaches for *H. fraxineus*. Many of these insights could also be transferable to other pathosystems, and the development of improved strategies for the study and management of forest diseases.

# One for all and all for one? How fungal pathogen combinations effect European beech trees and the stress marker FsPDF2

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Climate change-related stressors, including drought, heat, and storms, increasingly threaten Central European forests and increase their vulnerability towards pests and pathogens. European beech (*Fagus sylvatica L.*), one of the ecologically and economically most important tree species in Central Europe, is also experiencing dramatic loss of vitality, which is associated with abiotic stress and a complex of opportunistic fungal pathogens. The interplay between fungal agents within this complex and the host response is not well understood. Within the SiRT project, we investigate whether sulfur deficiency affects the stress physiology and defense capacity of European beech against (a)biotic stressors. In a controlled greenhouse experiment, three-year-old beech trees were inoculated with *Biscogniauxia nummularia*, *Neonectria coccinea*, and *Diplodia mutila*, which are prominent fungal examples within this disease complex and have frequently been detected in declining beech trees in recent years. The fungi were either inoculated individually or in all possible combinations. Disease severity was assessed based on necrosis development and wilting symptoms. Fungi were isolated from necrotic tissue, assigned to morphogroups, and selected isolates were identified by ITS sequencing followed by BLAST identification. In parallel, total protein content and the concentration of the plant defensin FsPDF2 were quantified in necrosis-adjacent tissue using the Bradford assay and an HRP-based sandwich ELISA.

Necrosis severity differed among treatments, with *D. mutila* inducing the largest necrotic lesions. Re-isolation success was also highest for single and combined infections involving *D. mutila*. In trees inoculated with *B. nummularia* and *N. coccinea*, numerous additional endophytic and saprobic fungal taxa were recovered from necrotic tissue. Total protein concentrations were significantly increased in D*. mutila*-inoculated trees compared with the control, whereas FsPDF2 concentrations did not differ significantly among treatments at the sampling date. These findings provide new insights into the interplay of multiple fungal agents in a complex tree disease, and serve as a basis for further investigations into the pathogen defense and stress physiology of European beech.

# The fungal-induced longevity gene F15E6.4 promotes fast paralysis of *Caenorhabditis elegans* during *Arthrobotrys flagrans* infection

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The nematode *C. elegans* is a well-studied model organism in many research fields such as neurobiology, development and immunity. It has several bacterial and fungal pathogens while it only relies on an innate immune system. After an infection, the expression of many immune response genes is induced. However, the role of single immunity genes is not very well understood. *A. flagrans* is a nematode trapping fungus that forms adhesive traps in low nutrient environments to catch and digest nematodes (Youssar et al. 2019). Fungal small-secreted proteins are important for infection (Emser et al. 2024). In addition, nematode small-secreted proteins play a role. We showed that the neuropeptide-like protein nlp-27 of *C. elegans* is induced during *A. flagrans* infection and enhances neurodegeneration and paralysis in *C. elegans* to facilitate infection and therefore faster availability of nutrients for the fungus (Pop et al. 2024). Here, we characterized the gene F15E6.4 of *C. elegans* and its role during infection. F15E6.4 is induced during fungal infection with *A. flagrans*, encodes 72 amino acids and localizes in the hypodermis of *C. elegans*, as well as at the trap site during infection. Overexpression of F15E6.4 prolonged the lifespan of *C. elegans* while it also slowed down larval development, decreased the number of offspring and also reduced the time until paralysis when the worm was trapped. F15E6.4 might slow down the metabolism of *C. elegans* to prolong the lifespan during fungal infection to increase the chance for survival. However, this represents a disadvantage for *C. elegans* in *A. flagrans* infection, since it will be paralyzed and killed faster. F15E6.4, like NLP-27 can be considered as endogenous virulence factor.

Youssar, L., Wernet, V., Hensel, N., Yu, X., Hildebrand, H.G., Schreckenberger, B., Kriegler, M., Hetzer, B., Frankino, P., Dillin, A., Fischer, R. (2019). Intercellular communication is required for trap formation in the nematode-trapping fungus *Duddingtonia flagrans*. PLOS Genet 15(3): e1008029.; Emser, J., Wernet, N., Hetzer, B., Wohlmann, E., Fischer, R. (2024). The cysteine-rich virulence factor NipA of *Arthrobotrys flagrans* interferes with cuticle integrity of Caenorhabditis elegans. Nat commun 15, 5795.; Pop, M., Klemke, A., Seidler, L., Wernet, N., Steudel, P.L., Wohlmann, E., Fischer, R. (2024). Caenorhabditis elegans neuropeptide NLP-27 enhances neurodegeneration and paralysis in an opioid-like manner during fungal infection. iScience 27.

# Molecular and rhizobiome-based insights into Verticillium wilt in hops

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Verticillium wilt caused by *V. nonalfalfae* is an emerging threat to hop production in the Hallertau, where lethal strains are increasingly displacing formerly mild populations. To elucidate disease-associated belowground processes, we combined qPCR-based pathogen detection in hop bines with amplicon sequencing of root, rhizosphere and bulk soil samples from infected and symptom-free hop plants. Across 105 field samples collected in 2023 and 2024, qPCR confirmed a clear predominance of lethal *Verticillium* strains, with 71 detections of lethal strains, only two detections of mild strains, and no *Verticillium* detected in 32 samples. In the rhizobiome study, plants of the susceptible cultivar Hallertauer Tradition were repeatedly sampled during two growing seasons, combined with visual disease assessment and qPCR-based classification of aboveground infection status. These samples provided a robust set of Verticillium-positive and -negative plants for downstream molecular analysis. DNA and RNA were extracted from rhizosphere and bulk soil fractions, and fungal and bacterial communities were profiled using ribosomal marker amplification followed by Oxford Nanopore sequencing and bioinformatic processing with Natrix2. *Verticillium* DNA was detectable in nearly all soil samples at the genus level, but species-level assignment remained limited by the available reference databases, which showed only about 95% sequence identity to the most similar entries. As a result, *V. nonalfalfae* could only be confirmed in a few cases, mostly in raw data, highlighting the need for a species-specific assay for complex soil matrices. Relative *Verticillium* abundance in the fungal rhizobiome ranged from 0% to ~30% and tended to be lower in Verticillium-negative samples, though the biological relevance of these differences remains unresolved without species specific detection. Comparative microbiome analysis identified taxa enriched in Verticillium-positive samples that may indicate infection or disease progression. Among fungi, *Fusarium* and *Coniochaeta* species were more frequent, while *Achromobacter* and *Pseudarthrobacter* were enriched among bacteria. No clear antagonistic taxa against hop wilt were detected. Future work will focus on expanding the *Verticillium* reference database, adapting the existing *V. nonalfalfae* specific qPCR assay to soil samples, and refining statistical analyses of rhizobiome shifts to clarify pathogen–microbiome interactions during early infection.

## Ecology of *Botryosphaeria dothidea*

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The cosmopolitan fungus *Botryosphaeria dothidea* (*Ascomycota*: *Dothideomycetes*) exhibits high ecological plasticity, transitioning between various plant-associated lifestyles. While it usually remains as an endophyte, climate-induced stressors such as drought can trigger a transition into an opportunistic pathogen, causing severe dieback, fruit rot, and irreversible wood damage in economically vital agricultural cultivars like fruit trees, nut trees or grapevine. Simultaneously, *B. dothidea* acts as a symbiont to ambrosia gall midges (AGMs; *Diptera: Cecidomyiidae*) within the tribes *Alycaulini, Asphondylini*, and *Lasiopterini*. Despite the strict host-plant specificity and global distribution patterns of these insects, their galls are universally lined with an internal mycelial layer dominated by *B. dothidea*. However, the evolutionary boundaries and genetic mechanisms driving these diverse lifestyles remain unresolved. To address this knowledge gap, this project scales up preliminary research, which was limited to 22 Czech strains (3 pathogenic, 19 gall-associated), by establishing an extensive collection of endophytic, pathogenic, and gall-associated isolates. DNA isolation and subsequent genome sequencing from this expanded collection will drive the first population genomics analyses of all three lifestyles to definitively test for cryptic speciation and underlying genetic differentiation. Furthermore, current work also focuses on optimizing sporulation induction, particularly among the challenging gall-associated strains, for use in controlled inoculation and virulence assays on Vitis vinifera to link specific fungal traits with host damage, thereby defining the evolutionary mechanisms of *B. dothidea*.

## Spp1-mediated processing of substrate proteins promotes plant defence suppression and virulence of fungal biotrophs

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In the smut fungus *Ustilago maydis*, the signal peptide peptidase (SPP) Spp1 is essential for plant defence suppression and establishment of a compatible biotrophic interaction with its host plant maize. SPPs are endoplasmic reticulum (ER)-resident intramembrane proteases, that cleave a subset of remnant signal peptides and transmembrane domain containing proteins, releasing them from the membrane for degradation or to exert a biological function. The conserved catalytic activity and generated cleavage products are crucial for the virulence function in *U. maydis*. Using a Co-IP/LC-MS approach from infected maize tissue, potential substrates of Spp1 were co-purified. Among these we identified the signal peptide (SP) of an effector protein, that is necessary for Spp1-dependent processing prior to effector secretion. Replacement of the Spp1-bound SP with an alternative SP abolished proper effector processing and significantly reduced virulence. In addition, expression of a predicted cleavage product is sufficient to partially rescue the virulence defect of the effector deletion strain. Another identified substrate is an ER membrane-anchored protein, that is required for virulence and depends on Spp1 for proper subcellular localisation. Direct transcriptional regulation via unfolded protein response elements (UPREs) links Spp1 function to biotrophic growth of *U. maydis*. In silico analyses suggest that the direct UPR-dependent regulation and its virulence function are specifically conserved in biotrophic fungi. Consistently, Spp1 is dispensable for virulence in the hemibiotroph *Verticillium dahliae* but essential for the biotroph *Sporisorium reilianum* to cause disease. Collectively, our data suggest that Spp1 mediates communication between biotrophic fungi and their host plants in a previously unknown manner.

# Investigating the role of mycobiomes for tree health in times of climate change

Michelle Thomaser

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Climate change increasingly threatens forest ecosystems by exacerbating heat and drought stress, thereby weakening tree vitality and increasing susceptibility to pathogens. Among these, fungal pathogens play a central role. *Diplodia sapinea*, typically an endophyte in pine species (*Pinus spp*.), has emerged as an opportunistic pathogen under changing environmental conditions, causing severe disease and tree mortality. While historically mostly present in the Southern Hemisphere, *D. sapinea* has recently spread widely across Europe. Studying the molecular basis of tree–pathogen interactions is still hampered by a lack of standardized methodologies for laboratory- and greenhouse-scale experiments. Despite its growing impact, forest pathology remains less well studied than its agricultural counterpart, lacking established model systems and transferable control strategies such as fungicide application. This project addresses these gaps through two main research foci. First, the infection mechanism of *D. sapinea*, which remains largely unknown, will be investigated using in planta microscopy. The methods for these approaches, including fluorescently labelled strains and laboratory-scale infection assays, have only recently been established by our group, enabling novel insights into the host–pathogen interaction. Second, the role of the pine mycobiome in modulating susceptibility to *D. sapinea* will be examined. This includes experimental deconstruction and reconstruction of the mycobiome, confrontation assays, and co-infection studies using synthetic communities. The goal is to identify potential protective functions of microbial communities and assess their relevance for biological control strategies. Together, this work aims to advance the understanding of forest pathogen dynamics under climate stress and to contribute to the development of sustainable disease management approaches in forest ecosystems.

## Competitors matter transmission upon death selects for a higher fungal load but not always an increase in virulence

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The contemporary idea of pathogen virulence evolution mainly stems from the trade-off hypothesis between transmission, virulence, and recovery rate. In spore forming parasites, however, growth rate is separated into two stages: vegetative growth, through which the host is exploited, and spore production, which is closely linked to transmission potential. This separation means that parasite growth may not be simply correlated with harm to the host. Furthermore, the fact that spores can persist in the environment has the potential to reduce or alter the costs of virulence through allowing parasites to sit and wait after hosts are killed. There are multiple mathematical models aiming to extend the trade-off hypothesis and understand virulence evolution in spore forming parasites. Some of these models predict that the timing of transmission (from living vs dead hosts) and whether parasites are in single or multi strain infections influence the trajectory of virulence evolution. These effects are also expected to be linked to toxin production, as burst killers often produce toxins to kill their hosts and transmit further. However, in spite of the substantial mathematical exploration of these questions, there are still only very few empirical tests. We established a model system to experimentally test the impact of parasite life-history and related trade-offs in vitro and in vivo. We carried out an evolution experiment to directly test this hypothesis by manipulating the point of parasite transmission (direct “Burst killing” and indirect “shedding” transmission) and using toxin-producing and non-producing strains and a mix of these two followed by metabolomic, genomic, and phenotypic analysis. We found that transmission by burst killing results in higher pathogen loads, but whether this results in increased virulence depends on the timing of toxin production in the starting strain and most critically whether the fungus is in a single strain or multi-strain infection. In addition, we shed light on further life-history trade-offs like vegetative growth and virulence and if they are correlated.

# Epigenetic regulation of the orsellinic acid gene cluster in *Aspergillus nidulans*

Paula Vargas-Trujillo, Peter Hortschansky, Volker Schroeckh, Axel Brakhage, Thorsten Heinzel

*Friedrich Schiller Universität Jena*

The orsellinic acid (ors) gene cluster of *Aspergillus nidulans* remains inactive under laboratory conditions. Co-cultivation of *A. nidulans* with bacteria of the genus Streptomyces induces orsellinic acid production through epigenetic remodeling. In this process, the SAGA/ADA complex acetylates histone 3 at the ors gene cluster, enabling binding of the transcription factor BasR and subsequent activation of orsellinic acid biosynthesis.
Given the pivotal role of BasR in ors cluster activation, we further investigated the underlying regulatory mechanisms. Using DNA pulldown assays, we identified a putative BasR binding sequence within the ors gene cluster. In addition, expression analyses and LC–MS studies revealed that SirE is the primary histone deacetylase (HDAC) repressing ors cluster expression.

Our findings provide new insights into the molecular regulation of bacterial–fungal interactions and uncover complex epigenetic mechanisms controlling silent secondary metabolite biosynthesis.

# Host-independent activation of biotrophy-associated gene expression in *Ustilago maydis*

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The hemibiotrophic fungus *Ustilago maydis* is the causative agent of the corn smut disease in *Zea mays* and switches from saprotrophic to biotrophic growth after successful mating and plant recognition. This morphogenetic and lifestyle transition is mediated by a comprehensive restructuring of the transcriptome, triggering a highly orchestrated virulence program including the stage-specific expression of virulence factors and effector genes. Although recent studies have provided important insights into transcriptome changes and stage-specific gene functions during biotrophic growth, detailed functional and biochemical analyses are hampered by low fungal biomass, plant-derived impurities and complicated downstream processing of biological material. To tackle these limitations, we sought to emulate the biotrophic gene expression program in axenic culture. To this end, previously identified transcriptional regulators were expressed in a stoichiometrically defined manner. Induction of these factors resulted in the upregulation of up to 70% of second-wave effectors accompanied by transcriptional activation of UPR markers and induction of upstream regulatory factors. On the morphological level, extensive hyphal growth in axenic culture and pronounced sensitivity to ER-stress on solid medium were observed. These findings indicate that the expression of the two factors alone is sufficient to induce ER-stress and to trigger the transition to biotrophic development. Strikingly, our data suggest a potential feedback-loop promoting sustained b-gene expression after plant penetration. In essence, we have designed a powerful tool that allows us to investigate plant-associated development and transcriptional programs under axenic growth condition. In proof-of-concept studies, we successfully used our expression system to study interactions between co-chaperones and their secreted substrates that are normally only expressed during biotrophic growth in planta. Thus, although completion of the *U. maydis* life cycle remains strictly dependent on its host plant, the analysis of the underlying intracellular processes has become substantially more accessible.

# Investigating the Origins of *Aspergillus fumigatus* Virulence in its Natural Compost Habitat

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*Aspergillus fumigatus* is an opportunistic human fungal pathogen widely occurring in environmental habitats such as compost heaps. It causes a wide range of diseases, ranging from allergic reactions to lethal invasive infections, depending on the host's immune status, and causes an estimated 4 million cases of invasive and chronic disease each year worldwide. Despite the absence of strong selective pressure for fitness in humans, this fungal pathogen has developed virulence traits enabling human infection. Emerging hypotheses posit that aspects of its environmental lifestyle, including interactions with other microbes, have shaped *A. fumigatus* virulence in human and mammalian hosts. The aim of this study is to investigate the ecological interactions of *A. fumigatus* in the compost niche with the ultimate goal of identifying key ecological functions and microbial partners that may have shaped its virulence in humans.

A total of twelve compost piles from different sites in Jena and the Rösl composting company in Leipzig, were sampled and resulted in the isolation of 29 bacterial and 27 fungal isolates. To assess their interaction with *A. fumigatus*, each isolate was tested pairwise with a bioluminescent reporter strain of *A. fumigatus*. The results obtained so far indicate that most bacterial and fungal isolates inhibit *A. fumigatus* growth, but to variable degrees. In future work, we plan to investigate the in situ gene expression of *A. fumigatus* within its native compost community to identify key ecological functions that allow *A. fumigatus* to overcome microbial competition, nutrient limitation and oxidative stress, and that may moonlight as virulence strategies during human infection.

# Development and Application of robust endpoint PCRs for the detection of causal agents of black rot canker in pome fruit - *Diplodia sp*. and *Diplodia bulgarica*

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Black rot canker in pome fruit is caused by fungi of the genus *Diplodia*. Only 2015 was *D. bulgarica* recognized as additional causal agent of black rot canker in domesticated apple. The global and European distribution pattern of D. bulgarica is uncertain, since it may has been misidentified in the past with *Diplodia* species already known as causal agents. To facilitate the detection of *D. bulgarica* and discrimination to other *Diplodia* species in context with black rot canker a robust endpoint PCR was developed to enable detection in DNA extracts from symptomatic wood. Two primer sets were established, one primer set targets *Diplodia* (Dip-PCR) as a genus and the second one is specific for *D. bulgarica* (Dbul-PCR). Inclusivity and exclusivity were tested with a broad set of *Diplodia* species, as well as other members of *Botryosphaeriaceae*, and other fungi commonly associated with diseases of woody plants and pome fruits. The primers were also tested on samples of 19 pome trees (apple and pear) with more and less pronounced symptoms of black rot canker, which did also undergo isolation assays. The PCR results and isolation results overlapped for 18 out of 19 samples for Dip-PCR and for all samples for Dbul-PCR.

# Unveiling the Impact of the Phyllosphere Mycobiome of Strawberry Plants on its Resistance Against Phytopathogenic Fungi

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Phytopathogenic fungi are responsible for substantial crop losses and challenge our ability to feed a growing population. The prevailing use of broad-spectrum fungicides, disrupts microbial biodiversity and contributes to environmental pollution. Therefore, the development of new, sustainable strategies is essential for the future of environmentally friendly farming. *Botrytis cinerea*, a widespread ascomycete pathogen of strawberries and many other crops, normally forms specialized infection structures on plant surfaces. Intriguingly, studies have shown that when *B. cinerea* spores encounter the saprophyte *Neurospora crassa*, they abandon infection-structure development and instead undergo germling fusion, assembling a non-infectious hyphal network. Because *N. crassa* and *B. cinerea* occupy different natural niches, we asked: does this kind of developmental reprogramming occur within naturally occurring phyllospheric communities? To answer this, we combined metabarcoding of the epiphytic mycobiome on strawberry leaves from four distinct sites with a culture-based approach. From our isolates, we specifically analysed 85 ascomycetes and screened them for intraspecific germling interactions. Remarkably, about one third exhibited germling fusion, revealing that this trait is broadly distributed across ascomycete lineages. We now want to test for interspecific interactions during germling fusion by co-cultivating them with isolated Botrytis strains. Co-incubation on detached strawberry leaves and fruits showed that some isolates have an effect on the infection symptoms commonly associated with Botrytis. To dissect these interactions under controlled conditions, we established an in vitro infection system using strawberry plants. By assembling defined synthetic mycobiomes on the phyllosphere before introducing phytopathogens, we can now quantify how specific fungal communities modulate pathogen virulence and host susceptibility. Taken together, our work extends the known taxonomic breadth of ascomycetes capable of germling fusion, identifies naturally co-occurring epiphytes that might impact *B. cinerea* infectivity on strawberry and provides a new system for exploring mycobiome- host dynamics. These findings lay the groundwork for further understanding the impact of epiphytic fungi in disease management.

# Regulatory mechanisms involved in the activation of the ors gene cluster in *Aspergillus nidulans*

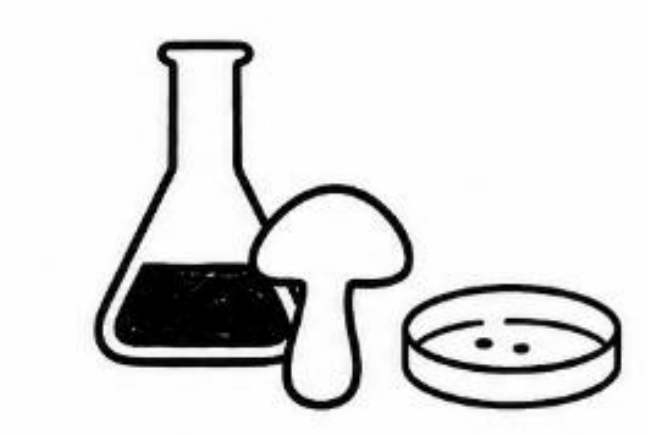
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*Aspergillus nidulans* is a filamentous fungus widely used as a model organism. It contains numerous biosynthetic gene clusters (BGCs) that are predicted to produce diverse secondary metabolites. However, many of these clusters remain silent under standard laboratory conditions, limiting their characterization. To activate silent BGCs, strategies such as microbial co-cultivation have been developed. A well-known example is the interaction between *A. nidulans* and Streptomyces species, which induces the activation of the ors gene cluster and the production of orsellinic acid. By mechanisms that are still needed to be elucidated, Streptomyces modifies the epigenetic landscape of *A. nidulans*. The SAGA/ADA complex in *A. nidulans* acetylates some Histone 3 lysine residues in the ors gene cluster, allowing the binding of the transcription factor BasR and the subsequent production of orsellinic acid. Given the pivotal role of BasR in ors cluster activation, we further investigated the underlying regulatory mechanisms. Using DNA pulldown assays, we identified a putative BasR binding sequence within the ors gene cluster. In parallel, expression analyses and LC–MS identified SirE as the primary histone deacetylase (HDAC) repressing ors cluster expression. SirE has previously been implicated in the regulation of development and secondary metabolism in *A. nidulans*. We employed a xylose-inducible sirE strain to investigate its regulatory function. Induction of sirE abolished orsellinic acid production and significantly reduced transcript levels of both ors biosynthetic genes and basR, whereas repression of sirE expression induced ors gene cluster activation. Similar effects were observed under conditions previously known to induce orsellinic acid production which are co-cultivation with *Streptomyces rapamycinicus* and treatment with Azalomycin F.

Our findings provide new insights into the molecular regulation of bacterial–fungal interactions and uncover complex epigenetic mechanisms controlling silent secondary metabolite biosynthesis.

# Fungal Biotechnology & Methods



## Total Biosynthesis of Specialised Metabolites from Waste

Russell Cox, Dr Jennifer Gerke, Ameer Syeed, Nele Peters

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*Aspergillus oryzae* and *Trichoderma reesei* have been engineered to produce a wide range of valuable specialised metabolites. Genes encoding biosynthetic proteins from fungi, plants and bacteria have been successfully incorporated into known and new biosynthetic pathways. In the case of *T. reesei* very low value substrates can be used to produce high value metabolites in a single fermentation.

## Transient transformation and episomal maintenance of the introduced plasmids in mushroom-forming fungi

Yoichi Honda, Daishiro Koshi, Dong Xuan Nguyen, Takehito Nakazawa, Moriyuki Kawauchi

*Kyoto University*

During introduction of transformation or genome editing systems into white-rot basidiomycete fungi, such as Pleurotus ostreatus and *Gelatoporia* (*Ceriporiopsis*) subvermispora, we have often encountered unstable drug-resistant transformants. In the beginning, we thought that they are background strains or abortive transformants, however more and more data provide evidence of extrachromosomal maintenance of the introduced plasmids and expression of not only drug-resistant marker gene but also others, including CRISPR/Cas9 system, for a short time. In this presentation, the authors show experimental data clearly indicating episomal maintenance of the introduced plasmids in these fungi. This phenomenon may be used for promoter assay, maker-less transformation or non-GM (Genetically Modified) genome editing without foreign DNA in other filamentous fungi.

# Overview about the chemical origin, biological triggers and biotechnological use cases of fluorescent mycelium from the genus *Ganoderma*

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Mycelium-based materials (MBC) are typically used as biobased and benign material alternatives for packaging, construction, insulation, and other applications. They are made from lignocellulosic plant residues (e.g. hemp shives, rapeseed straw, or wood chips) colonized by wood-rotting fungi such as *Trametes sp.*, *Pleurotus sp*. or *Ganoderma sp.*. Research on MBCs at Fraunhofer WKI has revealed a multicolored fluorescence of the mycelium and respecting fruiting bodies of several species from the genus Ganoderma.

The spatial and temporal change of the fluorescence on the surface of mycelium in solid-state culture on hemp shives depends on the specific cultivation conditions and fungal strains used. The intensive fluorescent colors are visible by eye or camera under UV light with a peak wavelength of 365 nm and point to underlying reactions of the fungus to multiple stressors. Using UV-induced visible fluorescence photography (UVIVF) adds a new layer of information in assessing mycelium for production of MBCs, edible or medicinal mushrooms and solid-state cultivation in general. The presentation therefore discusses the chemical origin, potential biological triggers, and possible technical applications of fluorescent mycelium.

Additionally, an overview of the ongoing artistic work of visual artist Malte Taffner in collaboration with Fraunhofer WKI project LuminousNetworks, utilizing UVIVF timelapse of statures made from fluorescent MBCs, will also be provided.

# Anaerobic gut fungi: molecular methods supporting detection, characterization, and activity monitoring for bioenergy applications

Veronika Flad, Diana Young, Katrin Stuer-Patowsky, Liren Huang, Nadine Lukas, Taiba Saleem, Claudio Kalbermatten, Sophia Felicitas Angelika Strobl, Bernhard Munk, Hans-Joachim Nägele, Sabine Marie Podmirseg, Michael Lebuhn, Christian Wurzbacher

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Anaerobic gut fungi (AGF), from the phylum *Neocallimastigomycota*, are highly efficient degraders of lignocellulosic biomass and promising candidates for bioenergy applications. Their practical exploitation requires robust molecular methods to detect, characterize, and monitor AGF during cultivation and to identify novel promising AGF in environmental samples. To address this need, we developed a set of complementary PCR-based assays, covering the workflow from environmental screening to process monitoring.

For environmental detection and community profiling, we established (RT)qPCR-based assays and sequence analyses specifically targeting the 28S rRNA gene of AGF. These assays were subsequently applied in simultaneous metabarcoding and quantification of AGF from environmental samples, providing insights into the community composition and revealing novel lineages (Young et al., 2022). To characterize newly obtained isolates in greater detail, we developed a second assay targeting the full ribosomal operon, enabling higher taxonomic resolution and comparative analysis across strains. In addition, we implemented an activity-monitoring assay for a GH6 exoglucanase, which allows tracking of the AGF transcriptional activity during cultivation and in bioreactors.

This integrated molecular framework accelerates the identification, selection and evaluation of promising AGF strains for lignocellulose conversion and other related processes providing a practical basis for advancing AGF in bioenergy applications and biotechnology.

References:

Young, D., Joshi, A., Huang, L., Munk, B., Wurzbacher, C., Youssef, N. H., Elshahed, M. S., Moon, C. D., Ochsenreither, K., Griffith, G. W., Callaghan, T. M., Sczyrba, A., Lebuhn, M., & Flad, V. (2022). Simultaneous Metabarcoding and Quantification of Neocallimastigomycetes from Environmental Samples: Insights into Community Composition and Novel Lineages. Microorganisms, 10(9), 1749. https://doi.org/10.3390/microorganisms10091749

# Microbial Dynamics and Physicochemical Changes During Banana Wine Fermentation Using Different Yeast Strains This study investigated yeast

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This study investigated yeast dynamics, physicochemical changes, microbial succession, and sensory quality during banana wine fermentation. Fermentation trials were conducted using both a pure, known yeast strain and naturally occurring microflora. Microbial counts, pH, temperature, titratable acidity, alcohol content, specific gravity, and sensory attributes were evaluated over a 144-hour fermentation period. Yeast populations increased significantly, reaching 2.52 × 10■ cfu/mL at 144 hours following a plateau between 48–120 hours. Total aerobic counts decreased progressively from 5.6 × $10^2$ to 2.0 × $10^2$ cfu/mL in treated samples, indicating microbial stabilization. Alcohol content varied among samples, with the pure yeast culture producing the highest yield (10.5%). A consistent decline in pH and specific gravity (1.090 to 1.040) was observed, while titratable acidity increased from 0.98 to 1.48 g/100 mL. Temperature fluctuations (25°C–24.5°C) reflected active fermentation metabolism. Identified microorganisms included *Saccharomyces cerevisiae*, *Bacillus spp*., *Lactobacillus spp*., and *Penicillium spp*. Sensory evaluation demonstrated significantly higher acceptability for the wine produced with the pure yeast strain in terms of aroma, taste, color, texture, and overall preference. These findings highlight the critical role of yeast strain selection in optimizing fermentation performance and sensory quality in banana wine production. The study provides valuable insights for improving fruit wine standardization and commercialization.

# Ac/Ds transposon mutagenesis enables mapping of non-essential genes in the rock inhabitant *Knufia petricola*

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Black fungi are a polyphyletic group of ascomycetes that evolved similar morpho-physiological adaptations to colonize extreme environments such as sun-exposed natural and human-made surfaces worldwide. Constitutive melanin formation, slow growth, and compact colony morphologies underpin their resilience but have long complicated molecular and genetic work, rendering black fungi a functional genetic "black box". A protoplast-based transformation procedure and CRISPR/Cas9-based genome editing in the rock-inhabiting species *Knufia petricola* (*Eurotiomycetes, Chaetothyriales*) were established. These enable multiple knockouts or knockins for complementation studies or live cell imaging, and thus efficient hypothesis-driven targeted mutagenesis. However, the unique ability of black fungi to colonize oligotrophic extreme environments remains poorly understood, and genes involved are unknown. To address this in *K. petricola*, a forward genetics tool was developed that uses the transposable elements Activator/Dissociation (Ac/Ds) from maize in a two-component system for random in vivo mutagenesis. The Ac transposase, fused to a nuclear localization signal, is expressed on demand by the Tet-on system from an intergenic region, while the Ds transposon, comprising a nourseothricin resistance (natR) cassette, disrupts a nutritional gene (either ade2, ura3 or ppt1) resulting in auxotrophic Ac/Ds starter strains. These strains are cultivated with doxycycline to induce transposition and then transferred to minimal medium for selecting prototrophic strains (revertants), in which the Ds transposon has been excised from the nutritional gene. Identification of Ds reinsertion sites in 115 individual natR Ds mutants revealed random transpositions and 30 non-essential genes. Current efforts include scaling up the procedure of mutant generation and a pilot transposon insertion sequencing (TIS) approach, involving the enrichment of Ds flanking sequences from a library of approximately 600 Ds insertion mutants, followed by Illumina sequencing and genome mapping. The overall goal is to generate saturated mutant libraries to enable systematic identification of essential genes under diverse culture conditions.

# FUNGA: a large language model based phenotype-oriented key gene mining platform for fungi species

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Fungi play pivotal economical and ecological roles in our society and ecosystem. Identification of functional genes controlling important agronomic traits and response to external stresses is one of the most important mission in fungi breeding and genetic studies. Many multi-omics techniques, emphasizing gene sequence variations, expression profile and epigenetic status, facilitates to generate massive potential gene exhibiting statistical significance; however, identification of key functional genes from those gene set is still time-consuming and challenging, largely due to the lack of a utility to mine candidate genes using large-scale gene and phenotype relationship information. Here, we present FUNGA (FUNctional Gene Annotation platform), an innovative tool for key gene mining platform for fungi species. Based on large language models (LLMs) with Retrieval-Augmented Generation (RAG) and Tool Calling technologies, FUNGA integrates multimodal gene and phenotype data, including sequencing data, gene function databases and academic literature, and employs vector embedding techniques to develop a phenotype-oriented gene mining platform. Taking advantage of phenotype-oriented gene mining method, FUNGA could not only comprehensively screen and accurately prioritize candidate functional genes from potential gene sets, but also unravel regulatory network among those genes. The evidence and deducing process for gene mining are illuminated by interactive visualizations and AI-generated summaries. More important, FUNGA exhibits robust expansibility to general fungi taxa, including model species yeast, edible and medicinal mushrooms and ascomycetes pathogens. To summary, as the first large language model based gene analysis utility for fungi species, FUNGA provide a tool for fungi gene function and interaction studies, as well as a phenotype-oriented platform for key gene mining for general fungi species.

# PRODUCTION AND CHARACTERIZATION OF BIOMASS AND EXOPOLYSACCHARIDE FROM EDIBLE MUSHROOM (*Lentinus squarrosulus*)

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Exopolysaccharides (EPS) produced by edible mushrooms are increasingly gaining attention due to their diverse bioactive and other valuable industrial applications. However, there is limited information on EPS production by *Lentinus squarrosulus* under submerged fermentation. The study investigated the production and physicochemical properties of fungal biomass and EPS produced by *L. Squarrosulus* cultivated under submerged fermentation. Two nutrient media, Potato Dextrose Broth (PDB) and Yeast Extract Broth (YEB) were used for cultivation over 21 days. The biomass yield, EPS concentration as well as the pH changes were measured at 3-day intervals. The highest EPS (0.46 ± 0.01 mg/ml) and biomass (6.83 ± 0.043 mg/ml) yields were achieved on day 9 in PDB compared to YEB which produced lower biomass and EPS. The characteristic polysaccharide functional groups, including β-glycosidic bonds of EPS was confirmed using Fourier-transform infrared (FT-IR). Functional analysis revealed high water solubility (54.55 ± 0.80%) and water absorbing capacity (91.40 ± 1.20%), and antioxidant assay showed strong antioxidant activity, with high active radical scavenging capacity of 87.41 ± 1.05%, equivalent to 0.415 ± 0.004 mg/mL gallic acid. Protein and carbohydrate profiles indicates that EPS was predominantly carbohydrate (62.26 ± 0.16 %). These results show that EPS from *Lentinus squarrosulus* possess favourable structural and bioactive attributes that strongly support its potential use in pharmaceutical and functional food-based applications.

# *Knufia petricola*: a potential platform for heterologous gene expression

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Black fungi represent a morphologically distinct group of Ascomycetes characterized by unusual cell division and formation of melanized compact colonies. *Knufia petricola*, a rock inhabiting member of the Eurotiomycetes, is non pathogenic and exhibits moderate growth in both surface and submerged culture. During the development of genetic tools for studying extremotolerance and biodeterioration, we identified features that make *K. petricola* an attractive host for heterologous gene expression.

The genome of *K. petricola* is compact (haploid, 28.1 Mb, ~10,000 genes) and reveals a remarkably reduced secondary metabolism, by lacking genes for toxin production. Instead, the fungus constitutively produces protective metabolites such as DHN melanin, carotenoids, and mycosporines, demonstrating the continuous investment of energy in metabolite synthesis rather than in biomass formation. A key advantage of *K. petricola* is its highly efficient homologous recombination machinery, resulting in very low rates of ectopic integration. This enables rapid and precise strain engineering – including multiplex editing of several loci – using plasmid based delivery of Cas9 and sgRNAs together with donor DNA carrying short homologous arms (≥ 40 bp). This system is complemented by a set of defined genomic insertion sites (safe harbor loci) that support reliable gene expression under the control of constitutive promoters or the inducible Tet on system. These include pigment biosynthesis loci, whose disruption enables visual screening of transformants and elimination of background pigmentation, as well as five experimentally validated intergenic regions. By using the P2A motif, multi gene constructs can be expressed from a single locus. Together, this toolbox enables controlled expression of multiple genes and thus the reconstruction of complex biosynthetic pathways in a stable host with minimal endogenous secondary metabolism, allowing clean product profiles and efficient metabolic flux redirection. Recently, we solved the last remaining bottleneck in genetic engineering, namely the generation of protoplasts from melanized cells, by transiently suppressing melanization using ascorbic acid.

Having validated the genetic toolbox using endogenous genes, we now aim to explore its potential for heterologous protein expression and pathway engineering and invite interested colleagues to collaborate in testing and applying this platform to their candidate genes and biosynthetic pathways.

# Chitin-binding properties of AFP from *Aspergillus giganteus*

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Climate change is promoting the emergence of pathogenic fungi, while fungicide overuse leads to antifungal resistance, threatening human health and food security [1-5]. Antifungal peptides offer a promising sustainable alternative to established antifungal drugs, with broad antifungal activity and low toxicity [6].

This study examines the chitin-binding properties of AFP from *Aspergillus giganteus*, focusing on regions with similarities of a hevein-like domain and bacterial chitin-binding sequence [7].

Recombinant production of a set of derivative proteins of AFP, with induced point mutations in putative functional regions were produced and will be compared in bioactivity and chitin-affinity. A susceptibility assay against *Aspergillus niger* revealed a putative antifungal region of AFP, and initial dissociation constant measurement, using a modified frontal affinity chromatography approach, revealed a low affinity against chitin, comparable to other plant lectins containing a hevein-like domain [8]. Furthermore, docking simulations of AFP to chitin presents a potential protein-ligand interaction at the targeted binding site.

This study gives a closer insight into the mode of action of AFP and the putative correlation of bioactivity and chitin-affinity. The modification of the frontal affinity chromatography approach offers a practical and high-throughput alternative to protein–ligand affinity measurement techniques that are limited by insoluble glycans, such as surface plasmon resonance.

[1] Seidel et al. 2024. https://doi.org/10.1016/S2666-5247(24)00039-9. [2] Williams et al. 2024. https://doi.org/10.1371/journal.ppat.1012219. [3] Nnadi et al. 2021. https://doi.org/10.1371/journal.ppat.1009503. [4] Gusa, Asiya. 2024. https://doi.org/10.1016/j.jbc.2024.106541. [5] Singh et al. 2023. https://doi.org/10.1038/s41579-023-00900-7. [6] Hassan et al. 2023. https://doi.org/10.3390/pharmaceutics15010072. [7] Hagen et al. 2007. https://doi.org/10.1128/AEM.02497-06. [8] Solanke et al. 2019. https://doi.org/10.1038/s41598-019-53815-w

# Molecular Adaptation of *Pycnoporus sanguineus* during Mycelium-Bound Composite Formation

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Mycelium-bound composites (MBCs) are promising bio-based materials for packaging, insulation, and lightweight construction, as they can convert lignocellulosic residues into functional biodegradable products. However, the fungal responses occurring within solid composite systems, and their influence on final material properties, remain insufficiently understood. Here, we combined material characterization with molecular analysis to investigate how the white-rot fungus *Pycnoporus sanguineus* adapts to beech wood and hemp shives during MBC formation.

Composites were assessed for colonization kinetics, microstructure, water uptake, compressive behaviour, pigmentation, transcriptomic adaptation, and protein extraction feasibility. Environmental scanning electron microscopy revealed substrate-dependent architectures. Beech composites developed a denser outer mycelial layer and a more compact internal structure, whereas hemp composites showed larger pore spaces and more extensive intermediate mycelium. These differences were reflected in performance: hemp supported faster and more uniform colonization but showed higher water uptake and lower compressive resistance, while beech produced stiffer composites with reduced water absorption. RNA-seq showed that *P. sanguineus* adjusts its transcriptional program according to carbon source and composite microenvironment. On defined media, lignocellulosic substrates induced expression profiles distinct from maltose and microcrystalline cellulose. In intact composites, transcriptomes separated by substrate type and oxygen-associated pigmentation state, suggesting that both substrate architecture and local oxygen availability shape fungal physiology during material formation. Protein extraction workflows were also established directly from composite material, and enzyme assays confirmed retained lignocellulolytic activity in extracellular extracts. These results show that substrate-dependent fungal adaptation shapes both the structure and performance of MBCs, providing a basis for optimizing substrate choice, cultivation conditions, and post-processing strategies for more reliable and sustainable mycelium-based materials.

# A multi-approach strategy for the discovery of novel natural compounds from the marine fungus *Asteromyces cruciatus* 763

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Marine-derived fungi represent a rich source of structurally diverse secondary metabolites, including steroids, peptides, terpenes, and alkaloids, many of which exhibit broad bioactivity [1] and significant pharmaceutical potential.

Within this specific group, the filamentous fungus *Asteromyces cruciatus* 763 (*Pleosporaceae, Ascomycota*), collected from the coast of La Jolla (San Diego, USA), has been identified as the natural producer of the cyclic non-ribosomal pentapeptide lajollamide A, whose structure was elucidated in 2012 by HPLC-MS and NMR [2]. Genome mining of *A. cruciatus* 763 revealed 29 putative biosynthetic gene clusters (BGCs) encompassing the lajollamide A BGC, which had been previously characterised [3]. However, the majority of the remaining BGCs appear transcriptionally silent under laboratory conditions, suggesting that *A. cruciatus* 763 harbours yet underexplored biosynthetic potential. In order to exploit this hidden metabolic capacity, we established a multi approach strategy including an OSMAC approach, heterologous expression and a CRISPR-Cas toolbox for the activation of silent BGCs from *A. cruciatus* 763. For the heterologous expression, six BGCs harbouring different type I PKS core genes from *A. cruciatus* 763 were selected and cloned individually under a strong constitutive eno promoter. Using PEG-mediated protoplast transformation , the recombinant plasmids were transferred into the auxotrophic *A. oryzae* NSAR1 strain , expecting to yield prototrophic transformants generated by multiple random ectopic integration of the introduced plasmid-DNA. Subsequent small-scale chemical screening of several transformants, followed by HPLC-MS and MS/MS, will be employed to detect novel marine-derived natural products with potential relevance for future drug development and biotechnological applications.

[1] Gonçalves, M. F., Paço, A., Escada, L. F., Albuquerque, M. S., Pinto, C. A., Saraiva, J. A., & Alves, A. (2021). Unveiling biological activities of marine fungi: The effect of sea salt. Applied Sciences, 11(13), 6008. [2] Gulder, T., H. Hong, J. Correa, E. Egereva, J. Wiese, J. Imhoff and H. Gross (2012). Isolation, Structure Elucidation and Total Synthesis of lajollamide A from the Marine Fungus Asteromyces cruciatus. Marine Drugs 10: 2912-2935. [3] Maier, H. (2025). Expanding the molecular tool box for the exploration of natural products from non-model ascomycetes. PhD work, Eberhard Karls Universität Tübingen, Germany

# Developing a heterologous production of azaphilone colourants in *Aspergillus niger*

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Colour is an immanent attribute of almost all items of our daily live. However, most colourants used are of synthetic origin. Due to political bans and social rethinking, the demand for natural alternatives has been growing.

Fungi produce a wide range of secondary metabolites (SMs), potentially serving as natural colourants in various industries. In contrast, parallel production of mycotoxins, a relatively slow growth rate or SM production under specific, potentially unfavourable conditions, make them irrelevant for an industrial production. A solution is the shift of production into an industrially established heterologous host, i.e. *Aspergillus niger*. *A. niger* has proven to be an efficient heterologous producer for non-ribosomal peptide- based SMs with high titers. Given the capacity of A. niger to produce diverse polyketides, we postulate that *A. niger* is also capable to provide high quantities of polyketide precursors, rendering it an optimal choice for heterologous gene cluster integration.

As a test case for this hypothesis, we aim to establish *A. niger* as a heterologous producer of azaphilones, a class of natural colourants within the yellow to red colour spectrum, from the genus *Monascus*. Therefore, refactoring of the *Monascus* azaphilone biosynthetic gene cluster (BGC) with an iterative integration approach for all genes encoding catalytic enzymes in the respective BGC was applied. Gene expression was validated on transcriptomic as well as proteomic level while product and intermediate formation was analysed by mass spectrometric methods. Thereby, we were able to observe heterologous production of azaphilones in *A. niger*, which is currently optimized and benchmarked against the native production in *Monascus* species. Results will be presented accordingly.

# Exploiting Wild-Type *Trichoderma* Diversity for Improved Wheat Straw Saccharification in Biogas-Relevant Biomass Conversion

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*TU Munich*

The growing demand for sustainable alternatives to fossil-based energy has intensified interest in the biological conversion of lignocellulosic residues. Wheat straw, an abundant agricultural and largely underutilized by-product, remains difficult to valorize due to its recalcitrant molecular structure. Filamentous fungi of the genus *Trichoderma* serve as key model organisms for lignocellulose degradation and as chief industrial producers for cellulases and hemicellulases.

In the present study, we systematically screened a diverse collection of *Trichoderma* isolates for endoglucanase, xylanase, and β-glucosidase activities, as well as saccharification efficiency on wheat straw. One *T. harzianum* and one *T. koningiopsis* isolate outperformed the benchmark strain *T. reesei* QM6a, showing up to 36.4% increased sugar release, accompanied by markedly enhanced β-glucosidase activity.

To investigate the molecular basis of this improved performance, genome sequencing and transcriptomic analyses during growth on wheat straw are currently underway. Preliminary analyses place the selected isolates in distinct phylogenetic clades within the genus *Trichoderma*, suggesting that evolutionary divergence contributes to functional differences in lignocellulose degradation. Comparative genomic exploration indicates variation in carbohydrate-active enzymes (CAZymes) repertoires, including glycoside hydrolase families such as GH3, GH5, GH7, GH10, and GH11, associated with cellulose and hemicellulose breakdown. These observations suggest that both phylogenetic background and CAZyme composition may contribute to the enhanced saccharification efficiency.

Overall, this study highlights the untapped potential of wild-type *Trichoderma* diversity and provides a framework for linking phylogeny and genomic features to lignocellulose degradation performance, supporting the development of improved biological pretreatment strategies for biogas-related biomass conversion.

# 3D imaging of plant-microbe and microbe-microbe interaction dynamics in transparent soil

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In soil and the plant rhizosphere fungi and bacteria are in constant interaction with their surroundings. Either with other organisms or by overcoming obstacles in the 3D organization of their habitat. Especially the physical structure of soil can be quite challenging for microorganisms because they have to deal with different particle textures and air- or water filled cavities of different sizes to get to their nutrient sources, hosts or interaction partners.

Since their growth, expansion or interaction behavior under such conditions is challenging to investigate by high resolution imaging techniques, we designed a microscopy 3D growth chamber filled with artificial transparent soil for confocal laserscanning microscopy and Lightfield 4D. For cultivation, transparent soils are a less artificial laboratory-based approach to study plant-microbe interactions. They are easily customizable and, unlike agar, have more soil-like physical properties as they form aggregates creating a three-dimensional but transparent growth medium. The chamber system can be used for both, cultivation and undisruptive microscopy of plant-microbe and microbe-microbe interactions allowing us to perform 3D imaging of rhizosphere-like structures as well as monitoring of colonization dynamics over time. So far, we established growth systems for tomato and poplar as plant models, several beneficial and pathogenic fungi as well as bacteria in different combinations and investigated their interaction with each other but also their growth behavior in a soil-like 3D pattern.

By further improving this chamber system as well as the soil particles we use for cultivation, we would like to make rhizosphere interactions of microbes more accessible by imaging techniques to predict plant-microbe and microbe-microbe interaction dynamics in their natural habitat.

# Optimization of citric acid fermentation using strain development strategies

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Citric acid is the most widely produced organic acid in the world and is manufactured almost exclusively using the filamentous fungus *Aspergillus niger*. This microorganism is capable of converting the carbon source into citric acid with an efficiency close to 100%. As a result, conventional citric acid fermentation has reached a high level of maturity, leaving limited scope for further improvements in the fermentation process itself. Cost reduction remains a key objective, which in this context may be achieved by optimizing the composition of the culture medium, adjusting process parameters, or engineering a genetically modified strain with improved performance.

One of the most critical factors influencing citric acid fermentation is the presence of $Mn^{2+}$. Even concentrations as low as 5 ppb (µg/L) can significantly reduce the citric acid yield, although this effect is critical only during the first 48 hours of fermentation. In *A. niger*, manganese uptake is mainly facilitated by the high-affinity NRAMP family transporter DmtA. Alterations in DmtA activity influence citric acid overflow as well as fungal morphology, thereby establishing a functional link between manganese transport and citrate production physiology. Manganese availability also affects the expression of the cexA gene, which encodes the major citrate exporter, with cexA expression being strongly upregulated under $Mn^{2+}$-limited conditions. Exploiting the relationship between cexA expression and manganese availability, it is possible to develop mutant *A. niger* strains capable of producing citric acid with the same efficiency as the parental strain under $Mn^{2+}$-limited conditions, while eliminating the need for manganese removal procedures such as potassium ferrocyanide treatment of the culture medium or ion-exchange purification. This approach has the potential to further enhance the economic and energy efficiency of the fermentation process.

# Phosphate availability influences biomass formation and the biomass-to-product ratio rather than itaconic acid accumulation in *Aspergillus terreus*

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Itaconic acid is an important bio-based platform chemical produced via large-scale submerged fermentation by the filamentous Ascomycete fungus *Aspergillus terreus*. Here, we examined the impact of initial extracellular phosphate concentration on itaconate production from D-glucose and D-xylose in optimized 6 L scale fermentations mimicking industrial settings. Phosphate availability primarily influenced biomass formation and the biomass-to-product ratio rather than directly affecting itaconate accumulation. Both lower- and higher-than-optimal phosphate concentrations decreased the volumetric and specific itaconate yields on both carbon sources; the highest productivity was observed at the optimized level of 0.1 g L−1. Expression of the aoxA gene, encoding the cyanide-resistant alternative oxidase, and alternative oxidase activity were inversely correlated with extracellular phosphate concentration. In contrast, total respiration rates and the overflow-associated pellet-type fungal morphology remained unaffected. These results show that phosphate mainly acts as a secondary modulator of itaconate fermentation through its influence on biomass formation in A. terreus. Although the initial catabolic pathways that lead from D-glucose and D-xylose toward itaconate differ, both pathways converge at the shared intermediates fructose-6-phosphate and glyceraldehyde-3-phosphate, after which carbon is processed through similar metabolic routes. This may explain why fermentation performances were qualitatively similar on D-glucose and D-xylose in relation to phosphate availability.

## Biomechanics of ascospores

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Ascospores are the sexual propagation units of ascomycetes. Depending on the lifestyle of the particular fungus, these spores have to withstand hazardous environmental conditions. The saprotrophic fungus *Sordaria macrospora* propagates on herbivore dung and discharges ascospores from fruiting bodies generated on this dung. Spores are taken up by herbivores and pass their intestinal tract. During this passage, the ascospores have to cope with acidic environment and high temperatures, and, importantly, they have to resist mechanical forces during rumination. We have measured the force *S. macrospora* ascospores can withstand using atomic force microscopy. Comparing melanized wild type spores and reddish-brown as well as albino spores, we find that melanin has a strong impact on ascospore mechanics. We further provide data on the cell wall differences of ascospores from spore color mutants using transmission electron microscopy.

# Fungal Pigments as Sustainable Colorants and Sources of Bioactive Compounds

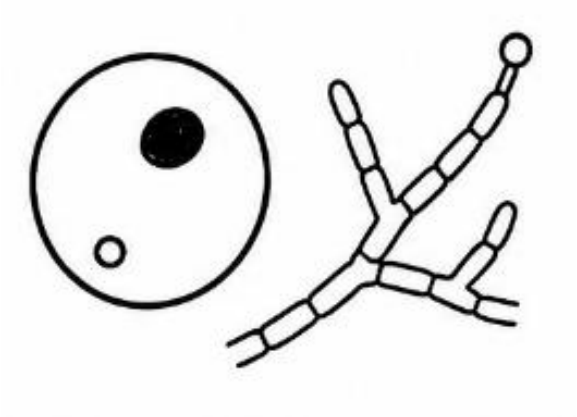
Janette Miled, Miriam Große, Hedda Schrey, Harry C. Evans, Nattawut Boonyuen, Jennifer Luangsa-ard, Umpawa Pinruan, J. Philipp Benz, Tian Cheng and Marc Stadler

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Fungi produce a wide range of secondary metabolites, including chemically diverse pigments such as azaphilones, xanthene derivatives, and quinone-derived pigments [1, 2]. Beyond their coloration properties, fungal pigments can exhibit biological activities, as reported for the antibiofilm-active azaphilone rubiginosin C, while other pigments, such as the red monascin, have been investigated as natural colorants [3, 4]. These examples highlight the potential of fungal pigments for applications in infection research and as sustainable alternatives to synthetic colorants. The aim of this project is therefore to investigate the chemical diversity of pigment-producing fungi and characterize their pigments regarding biological activity and potential applications. At the Helmholtz Centre for Infection Research (HZI), cultivation conditions are optimized for pigment production, followed by isolation and chemical characterization of the pigments and investigation of their biological activities. In parallel, the Technical University of Munich (TUM) uses genetic engineering of *Hypoxylon* spp. to increase pigment yields, while the National Center for Genetic Engineering and Biotechnology (BIOTEC) in Thailand produces mycelium-based leather for coloration with pigments isolated and characterized at HZI. Three pigment-producing fungi from different taxonomic groups and ecological niches were initially selected at HZI: the entomopathogenic ascomycete *Samsoniella scoliopterygis*, the saprobic ascomycete *Hypoxylon fragiforme*, and the saprobic basidiomycete *Terana coerulea*. All three fungi were screened in different cultivation media, followed by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) analysis of the extracts. Current work focuses on *S. scoliopterygis*, for which brown rice medium (BRFT) was selected for scale-up based on the initial screening. HR-ESI-MS analysis revealed the yellow pigments farinosone A, B and D as well as several pigment-related isomers. Farinosones A, B and D have previously been reported to exhibit activity against *Staphylococcus aureus*, while biofilm inhibition has been reported for farinosones A and D [5]. The investigation of *S. scoliopterygis* provides first insights into the secondary metabolite profile of this recently described species. Future studies will extend the chemical investigation to additional pigment-producing fungi to identify pigments with potential relevance to infection research or as sustainable colorants.

[1] Shi, Y. et al., Mycology, 2024, 15(3), 283–321. [2] Elkhateeb, W. et al., Appl. Microbiol., 2023, 3(3), 735–751. [3] Zeng, H. et al., J. Fungi, 2023, 9(7), 726. [4] Manzano, K. et al., Food Biosci., 2026, article 108875. [5] Toshe, R. et al., Beilstein J. Org. Chem., 2025, 21(1), 327–339

# Cell Biology & Development



# The conserved translational repressor Ssd1 controls cell wall plasticity during endosomal mRNA transport

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Pathogenic fungi depend on precise polar growth and the yeast-to-hyphal transition, requiring the strict spatiotemporal control of gene expression. A central mechanism to achieve this is the long-distance transport of mRNAs along microtubules via endosomal hitchhiking. While the transport of messenger ribonucleoprotein (mRNP) complexes is well established, the specific regulation of mRNA fate during transit is poorly understood. Our research highlights the vital role of the highly conserved translational repressor Ssd1 within these shuttling mRNPs.

In the model fungus *Ustilago maydis*, live-cell imaging and interactome analyses reveal that Ssd1 undergoes processive bidirectional movement and associates with endosomal mRNPs in an RNA-independent manner. It is specifically recruited to the complex via the RNA recognition motif RRM1 of the endosomal transporter. Transcriptome-wide analyses demonstrate that Ssd1 directly targets a subset of mRNAs encoding essential cell wall-remodeling enzymes, such as the β-1,3-glucan synthase Gsc1, by binding to conserved motifs clustered in their 5' UTRs. Phenotypic analyses confirm that both the overexpression and depletion of Ssd1 cause hypersensitivity to cell wall-degrading enzymes and disrupt overall cell wall integrity, underscoring its necessity for proper hyphal expansion. Ultimately, Ssd1 ensures that these specific cargo mRNAs remain translationally repressed until they reach the growth pole.

Building on these insights into spatiotemporal translation control, we are currently investigating the conserved regulatory mechanisms of Ssd1 in the human fungal pathogen *Candida albicans* utilizing Yeast Three-Hybrid (Y3H) systems. Furthermore, to evaluate the functional conservation of this critical regulatory network, we are performing cross-species complementation assays by expressing the *C. albicans* Ssd1 ortholog in *U. maydis* ssd1 mutant backgrounds.

## Structural characterisation of *Neurospora crassa* eisosomes

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MCC/eisosome complexes are stable plasma membrane domains that regulate membrane organization, protein trafficking, and stress responses in fungi [1,2]. The Membrane Compartment of Can1 (MCC) is enriched in specific lipids and transporters, while eisosomes are large protein assemblies that scaffold membrane invaginations.

In *Neurospora crassa*, Lsp1 was established as an eisosomal marker due to its predominantly punctate plasma membrane localization [3]. Its distribution changes during development, with the highest density in germinating spores. Distinct localization patterns in hyphae emerging from sexual versus asexual spores further suggest developmental regulation of eisosome organization [4].

To investigate eisosomal dynamics, GFP- and RFP-tagged constructs of core eisosomal proteins were generated by CRISPR-mediated integration at their native loci. Particular focus was placed on Lsp1 and Div23, two BAR domain-containing proteins implicated in plasma membrane furrow formation. Despite 58% sequence similarity, Div23 displays a localization pattern distinct from Lsp1. While Lsp1 is largely confined to punctate plasma membrane structures, Div23 also exhibits a substantial cytoplasmic fraction and colocalizes with Lsp1 only during specific developmental stages.

To examine protein interdependency, loss-of-function mutants were generated in the double-fluorescent strain background. These strains are being analyzed for changes in protein localization, plasma membrane furrow formation, and developmental or morphological phenotypes. In parallel, a CFP-tagged version of Nce102 is being introduced into the same strain. As Nce102 stabilizes eisosome-associated plasma membrane furrows in yeast, its localization may provide further insight into eisosome assembly and maintenance.

1. Moseley JB. Curr Biol. 2018;28:R376–R378. 2. Kolláth-Leiß K, Kempken F. In: The Mycota XV: Physiology. Springer; 2017. p. 119–130. 3. Kollath-Leiß K, et al. Eukaryot Cell. 2014;13:1051–1063. 4. Kollath-Leiß K, et al. J Fungi. 2023;9:147.

# Autophagy in the filamentous fungus *Sordaria macrospora*: The roles of SmATG11 and SmATG17 in selective and non-selective degradation

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Selective autophagy is a conserved subcellular process that uses receptors to target specific cargos for degradation in the vacuole. In the filamentous ascomycete *Sordaria macrospora*, neighbor of BRCA1 (SmNBR1) was identified as a receptor for the selective autophagy of peroxisomes (pexophagy). However, little is known about the interactions of SmNBR1 with other proteins. Thus, to identify SmNBR1-interactors we employed the Biotin Identification (BioID) method. Hereby, SmNBR1 is fused to a promiscuous biotin-ligase, that labels neighboring proteins with biotin. Biotin-labelled proteins are then enriched by affinity purification and identified by liquid chromatography coupled to mass spectrometry (LC MS). Among the identified candidates, we found SmATG11, a homolog of the Saccharomyces cerevisiae autophagy protein Atg11. Since ATG11 homologs in diverse organisms are scaffolds crucial for various selective autophagy pathways, SmATG11 is a reasonable SmNBR1 interactor. While S. cerevisiae Atg11 was thought to be exclusively involved in selective autophagy, with Atg17 fulfilling the equivalent role in non-selective bulk autophagy, recent studies suggest a less rigid functional separation. Moreover, ATG11 homologs in Homo sapiens and *Arabidopsis thaliana* handle both, selective and bulk autophagy, while ATG17 homologs are absent. Thus, besides studying SmATG11, we are also interested in the function of SmATG17. We discovered that *S. macrospora* encodes two variants of Smatg17, which we termed Smatg17a and Smatg17b. According to our in-silico analysis, the Smatg17a locus is highly conserved and encodes a full-length protein, while the Smatg17b locus is less conserved and produces a non-functional transcript. Therefore, we assume Smatg17b to be a pseudo-gene and mainly focus on the functional analysis of Smatg11 and Smatg17a. Interestingly, both single gene deletions only have mild effects on vegetative growth and sexual development, strongly contrasting the phenotypes of other autophagy mutants. Based on the close connection of ATG11 and ATG17 homologs in other organisms we therefore hypothesize functional redundancy of the genes in *S. macrospora*. Thus, we are currently constructing a Smatg11/Smatg17a double deletion strain. We believe this work is valuable not only for elucidating interaction networks in selective autophagy of *S. macrospora* but also for advancing our understanding of the evolution of selective and non-selective autophagy across species.

# Homeodomain Transcription Factor is Essential for Toxocyst Formation in the Oyster Mushroom *Pleurotus ostreatus*

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Predation is one of the common ecological interactions across various life forms, with fungi exhibiting diverse strategies to prey on nematodes. The basidiomycete *Pleurotus ostreatus* (oyster mushroom) has evolved toxin-containing toxocyst structure to immobilize and consume nematodes as a nutrient source, particularly under nutrient-limited conditions. However, the molecular mechanisms governing toxocyst formation remain largely unclear. In this study, we conducted a forward genetic screen to identify mutants deficient in nematode paralysis, referred to as lot mutants (Lost Of Toxicity), and identified a homeodomain transcription factor HDT1 (HomeoDomain transcription factor for Toxocyst development) required for toxocyst formation. Deletion of the hdt1 gene mirrored the lot mutant phenotype, with normal growth but a defect in toxocyst development. Confocal images showed that the GFP fused Hdt1 are localized in the hyphae with toxocyst, further suggesting the role of regulating toxocyst development. Comparative RNA-seq analysis between the hdt1 mutant and the wild-type strain under nutrient-limited conditions revealed significant differential expression, with approximately 700 down-regulated and 280 up-regulated genes. Gene ontology enrichment analysis indicated that most of the down-regulated genes are associated with heme binding, ion binding, and oxidoreductase activity. Notably, two small secreted peptides (SSPs) with intrinsic disordered regions and two lectins were significantly down-regulated in the hdt1 mutant. Fluorescence protein labeled SSPs (Spc1 and Spc2) and lectins (Lec1 and Lec3) showed that they mostly colocalized together within the hyphae and toxocysts. Deletion of LEC1 impaired toxocyst formation. However, deletion of these SSPs only partially impeded toxocyst formation, suggesting the possibility of functional redundancy of the SSPs.

# Ras Signalling in the Basidiomycete *Coprinopsis cinerea*

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Ras proteins are highly conserved small GTPases that regulate fungal growth, morphogenesis, and development through the modulation of signalling pathways. These proteins function as molecular switches by cycling between an active GTP-bound state and an inactive GDP-bound state, a process controlled by Guanine Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs). Perturbation of this regulatory cycle can result in profound developmental and morphological defects. Constitutively dominant-active GTP-bound (RasVal19;) and dominant-negative GDP-bound (RasAsn24) mutant alleles were generated from a cloned ras gene from *Coprinopsis cinerea* by changing codons within GTP-GDP binding site. The RasVal19 and RasAsn24 alleles were introduced into different monokaryotic strains, e.g. AT8 (A43 B43) and FA2222 (A3 B6). Transformants exhibited distinct alterations in colony morphology relative to wild-type strains, consistent with Ras-associated phenotypes described in other fungal systems. Expression of RasVal19 resulted in strongly reduced radial growth accompanied by increased colony compactness, whereas RasAsn24 transformants displayed markedly impaired growth, sparse aerial mycelium, and reduced hyphal thickness. Our investigations focus on comprehensive phenotypic characterization of these monokaryotic transformants, including analyses of vegetative growth, hyphal morphology, asexual sporulation, sclerotia and chlamydospore formation, and developmental responses to light and dark conditions. Transformants can be mated with untransformed strains or with transformed strains of equal or opposite ras mutation. Systematic mating assays involving mutant and wild-type transformants are conducted to evaluate the roles of Ras signalling in mating-related processes, including hyphal fusion, nuclear exchange and migration, and clamp cell production. Dikaryons will be analysed for colony architecture, dormant structure formation, fruiting body development, and responses to environmental regulatory cues. Initial fruiting tests with rasval19 dikaryons indicate formation of bonsai fruiting bodies, defects in meiosis, with variably no or reduced numbers of basidiospores per mushroom cap. Spores have only small sizes, are often unstained and rarely stained. Collectively, these studies will provide further insight into the functional roles of Ras-mediated signalling in the regulation of fungal morphogenesis and developmental differentiation in *C. cinerea*.

# From single cells to biofilms: nutrient-dependent growth dynamics, morphology, and mechanics in *Knufia petricola*

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Rock inhabiting black fungi colonize both natural (rocks) and anthropogenic (monuments, façades, solar panels) surfaces. Their resilience under extreme conditions highlights their ecological role in desert ecosystems and their contribution to rock and material weathering. These properties make them relevant to geomicrobiology, material science, and astrobiology. However, the link between single cell growth dynamics and the resulting biofilm morphology and mechanics remains poorly understood. Among black fungi, *Knufia petricola* has emerged as a genetically and experimentally tractable model system. By combining biofilm assays, biomechanical measurements, and single cell microfluidics we establish and present a multiscale experimental framework to quantify growth dynamics, morphology, and mechanical properties from individual cells to mature biofilms. These studies are conducted under defined nutritional conditions [i.e. glucose vs. sucrose as single carbon (C) source and nitrate vs. ammonium as single nitrogen (N) source]. Biofilms grown across a range of C:N ratios displayed maximal growth at C:N of 60, while the nitrogen source strongly influenced colony morphology, mechanical behavior and the mode of cell division. Nitrate enhanced filamentous growth at the colony margin and pronounced surface structuring compared to ammonium. Cells divided predominantly by budding under nitrate conditions, whereas ammonium promoted increased meristematic cell division. Importantly, we successfully applied mechanical assays originally developed for bacterial biofilms to *K. petricola*, demonstrating their suitability for melanized fungal biofilms. These measurements revealed that nitrate grown biofilms were significantly stiffer and more cohesive than ammonium grown counterparts, linking nutrient dependent cellular growth programs to biofilm mechanics. Using single-cell microfluidics, we quantified how nutrient conditions modulate cell cycle dynamics and branching behavior. Our results revealed that increasing the C:N ratio from 0.6 to 60 caused shorter cell cycles and more branching, and no branching without supplemented C and N. Overall, K. petricola can mitigate nitrogen pollution in both agricultural and urban settings and may serve as a pollution indicator. Further the nitrogen source influences fungi–material interactions. Our results provide quantitative data for individual based models linking single cell growth to biofilm formation during surface colonization.

# The role of transcription factors in the development of complex multicellular structures in the ascomycete *Sordaria macrospora*

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The filamentous ascomycete *Sordaria macrospora* is used as a model organism to study the development of complex multicellular structures, namely fruiting bodies, which only occur during sexual development. Self-fertilization (homothallism) and the absence of an asexual propagative cycle are among the advantages that *S. macrospora* offers in the study of fruiting body development. Previous studies indicated that a genetic network regulates the course of fruiting body development through transcription factors and chromatin modifiers. However, many factors contributing to the multicellular development of sexual structures of filamentous ascomycetes remain unknown. One such factor contributing to this developmental cascade is the transcription factor PRO44. Deletion of pro44 leads to a developmental arrest at the protoperithecial stage, and additionally, a spatial mislocalization of the fruiting body precursors submerged in the agar. The aim of the study is to unravel the function of PRO44 by identifying interaction partners through the use of TurboID proximity labeling and determining transcriptional profiles of the different cell types involved in fruiting body morphogenesis by establishing single-cell or single-nucleus RNA-Seq. TurboID allows for the identification of interaction partners in vivo through biotin-labeling by a promiscuous biotin-Ligase in close vicinity to the protein of interest. The biotinylated proteins are enriched and subjected to LC-MS.

In previous studies, transcriptomic data were generated for various developmental stages, such as vegetative mycelium and whole protoperithecia as well as perithecia. In addition, some developmental mutants were also subject to RNA-Seq analysis. Morphologically, at least 13 distinct specialized cell types were distinguished during the perithecia development of *S. macrospora*. For single-cell RNA-Seq, mycelium cultivated under conditions that allow for sexual development is subject to protoplasting. One main obstacle of this method is that, as development progresses, cell wall stability increases, which leads to insufficient preparation of protoplast. Alternatively, for the later developmental stages, the isolation of nuclei might be employed by mechanical disruption of the cells, the presence of a suitable buffer, followed by gradient centrifugation. Preliminary data will be presented

# Investigation of the membrane damage response in the ascomycete fungus *Neurospora crassa*

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Maintaining the integrity of the plasma membrane is essential for all living organisms. Therefore, mechanisms have evolved to quickly and effectively repair damage caused by mechanical or chemical stressors. These repair mechanisms commonly include the detection of an intracellular Ca2+ increase, the reorganization of the cytoskeleton, and the targeted recruitment of proteins to the site of the injury.

To understand the fundamentals of membrane repair in fungi, we are investigating the proteins involved in membrane repair in the ascomycete *Neurospora crassa*. Our earlier studies demonstrated the involvement of the ALG-2 homolog PEF1, as well as the annexins ANX14 and ANXC4, in the repair of mechanically or drug-induced membrane wounds, with some of these proteins performing overlapping functions. Live-cell imaging revealed the accumulation of GFP-tagged PEF1 and ANX14 at the wound site originating during aberrant cell-cell fusion.

An accumulation of ANXC4 at the wound site can only be observed in the absence of the protein ANX14, suggesting a compensatory function for this protein. In general, mutant strains lacking the annexins exhibit a higher lysis rate in cases of failed cell-cell fusion compared to pef1 mutants, whereas pef1 deletion strains exhibit a higher lysis rate compared to annexins when treated with the pore-forming saponin (PFT) tomatine. Furthermore, we are investigating the role of actin in membrane repair. Cells are treated with the actin polymerization inhibitor latrunculin A before membrane damage is induced by the addition of tomatine. Subsequent protein localization revealed that ANX14 continues to be recruited to the plasma membrane, whereas PEF1 is no longer recruited to the plasma membrane. This result suggests that both proteins are involved in independent repair mechanisms. Further experiments aim to identify and characterize potential interaction partners of the membrane repair proteins in order to improve our understanding of the molecular basis of membrane repair. This will involve labeling potential interaction partners in vivo via proximity labeling using a modified biotin ligase and identifying them through mass spectrometry. Furthermore, potential membrane repair proteins will be identified in a candidate-based approach. Respective gene knockout mutants of *N. crassa* will be examined for increased sensitivity to mechanically or chemically induced membrane damage.

# Cellular Localization and Function of Striatin in *Schizophyllum commune*

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Striatin is a conserved scaffolding protein that anchors signaling complexes to membrane microdomains via a caveolin-binding site, thereby linking lipid raft organization to downstream cellular responses. These specialized, sterol- and sphingolipid-rich membrane domains facilitate the interaction of signaling molecules. While striatin has been well studied in higher eukaryotes, its role in development and intracellular signaling remains poorly characterized in the basidiomycete fungus *Schizophyllum commune*.

We investigated the cellular localization of striatin using the fluorescent protein eGFP and evaluated its function through targeted gene deletion (Δstr1). Deletion of striatin in the dikaryotic stage resulted in morphological defects in fruiting bodies, including aberrant primordia formation.

RNA sequencing and RT-qPCR analyses showed significant upregulation of fruiting body-associated transcription factors in the mutant, suggesting that striatin affects developmental gene expression. In addition, loss of membrane-associated striatin altered hyphal growth patterns, leading to irregular branching. Differential gene expression analysis further linked striatin function to downstream pheromone response signaling.

These findings establish striatin as a regulator of morphogenesis and signal transduction in *S. commune*.

# Mitochondrial inheritance and developmental regulation in *Coprinopsis cinerea*

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Four different types of circular mitochondrial genomes of the basidiomycete *Coprinopsis cinerea* were described in the past (H, J, P and S), with sizes between 36 to 42 kb distinguished by different indels. The two so far sequenced 42 kb long mtDNAs from strains Okayama 7 and AmutBmut are of the H type, with a 1.2 kb insertion between genes cox1 and rpo. The two DNA molecules are 99.96% identical to each other, with a total of 8 places of minor DNA similarity. In crosses between two compatible strains of *C. cinerea* of different mating types, mtDNA is maternally inherited unlike the nuclear genome which vice versa invades the foreign mating mycelium. Matings therefore can potentially result in two genetically different dikaryons. AmutBmut is a self-fertile homokaryon with a single haploid nucleus type that by mutations in the two mating type loci A and B can produce fruiting bodies with sexual basidiospores. Further mutations in other genes however could again disrupt the ability to develop fruiting bodies. Strain Proto159 is one of such mutants. This homokaryotic derivative was obtained by asexual spore (oidia) protoplasting and regenerating from AmutBmut. Like the parental strain, it has the dikaryon-typical clamp cells at its hyphal septa, but has reduced aerial mycelium despite of light-induced aerial oidia formation. Unlike AmutBmut, Proto159 produces a brown pigment (melanin) by laccase action that stains liquid and solid culture media dark. Moreover, it is blocked in fruiting at the very initial stage of localized hyphal branching and aggregation for fruiting body development. Proto159 has been mated with compatible self-sterile monokaryons. Dikaryons are readily formed in the crosses at the sides of the typical self-sterile monokaryons. Such dikaryons are non-staining and give rise to fruiting bodies with sexual basidiospores, indicating that any nuclear mutations in fruiting would be recessive. On the Proto159 sides of crosses, mated dikaryons are more difficult to obtain. Morphologies of potential dikaryons appear less pronounced than those obtained from the self-sterile monokaryon side, possibly by an interplay of resident mtDNA with incoming nuclear genomes. Ongoing analyses aim to provide insights into the interplay between mitochondrial inheritance and nuclear genetic factors governing fungal morphogenesis in C. cinerea.

# Metabolic cooperation between *Coprinopsis cinerea* and *Gongronella butleri* w5 facilitates sucrose utilization under coculture conditions

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Fungi coexist in shared environments where they interact in complex ways, either competitive or cooperative. They may compete for space and nutrients by modifying their own or other mycelial morphology or by secreting bioactive compounds like pigments, toxins, antifungal proteins, and enzymes. However, fungal interactions are not exclusively antagonistic. In some systems, they establish syntrophic relationships in which the metabolic activity of one fungus generates substrates that support the growth of another. One important form is carbon-source syntrophism, whereby microbial communities collectively exploit substrates that individual species could not utilize independently. As chemoorganotrophic organisms, fungi rely on fixed organic compounds as sources of carbon and energy, for instance glucose and fructose or the disaccharide sucrose. Utilization of sucrose requires its hydrolysis into glucose and fructose, an invertase catalysed reaction, which irreversibly cleaves the glycosidic bond between the two monosugars. *Gongronella butleri* and *Coprinopsis cinerea* both lack extracellular invertases to break down sucrose. *G. butleri* has an expressed transporter gene that allows uptake of the sucrose for intracellular conversion into glucose and fructose. This fungus consumes glucose but not fructose, which is excreted and becomes available for growth of *C. cinerea*. This suggests that *C. cinerea* may be unable to independently hydrolyze sucrose, which limits its growth in sucrose-containing media. Traces of maltose however induce a transporter that also takes up sucrose. It is then hydrolyzed by an intracellular invertase into its monosugars, both of which are used by *C. cinerea*. The coexistence of fungal species with distinct metabolic capabilities raises also competitive interactions by which *G. butleri* defeats *C. cinerea* through ROS formation. *C. cinerea* reacts on this stress with laccase Lcc9 production, induced by HBA (4-hydroxybenzoic acid) which is constitutively provided by G. butleri. Reactions in fungal coculture originally were studied in liquid medium, with *C. cinerea* separated from *G. butleri* by dialysis tubing. On sucrose-containing solid medium inoculated with the two fungi at around 4 cm distance, cooperative growth promotion of *C. cinerea* by *G. bulteri* can likewise be demonstrated. Simultaneously, 1 to 2 mm-wide growth-free barriers form between the colonies as zones of mutual inhibition, which also indicate strong antagonistic reactions.

# Cleansing of developing primordia of *Coprinopsis cinerea* by droplets containing defense proteins secreted by the pileipellis

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Exudation is a widespread but poorly understood phenomenon in fungi. In mushrooms, extracellular droplets are increasingly recognized as biologically active excretions rather than passive releases. In the coprophilous *Coprinopsis cinerea*, we observed a characteristic exudation process throughout fruiting body development. Developing primordia excrete translucent droplets from the cap outermost tissue layer (pileipellis). These droplets slowly roll downward along chains of veil cells, accumulate at the cap-stipe junctions, and eventually at fruiting body maturation flow down to the stipe base at the mycelium taking on a pale-yellow coloration. The directional movement of the droplets suggests a functional role analogous to a "lotus effect," in which water-associated surface activity contributes to cleaning and protecting exposed tissues. Droplet contact angles on mycelium decreased compared to water by a higher level of hydrophobicity. Proteomic analyses identified water-surface-active proteins, which may reduce surface tension and promote slow droplet mobility, thereby facilitating the removal of particles, microbial contaminants and pathogens from sensitive reproductive tissues. In total, 318 proteins were identified in the droplets, including 142 proteins carrying N-terminal secretion signals. A conserved core set of 144 proteins was present in all investigated stages of primordia formation (P1–P5), indicating a stable exudate-associated secretome during fruiting body development. Numerous proteins, often from the same families, with predicted or known defense functions have been identified. Prominent defense-associated proteins included galectins, ricin-family proteins, and copsins. Galectins are carbohydrate-binding lectins implicated in anti-predator interactions, whereas ricin-family proteins belong to ribosome-inactivating toxins with potential insecticidal or fungicidal activity. Copsins are defensin-like antimicrobial serine protease inhibitors and will protect against bacterial colonization in nutrient-rich dung habitats. Multiple members of various other multigene families, including from the subtilisin, fungalysin, and other protease families, laccases, may represent novel antibacterial, antifungal, insecticidal, or nematicidal factors. Together, our results indicate that exudate droplets in the coprophilous *C. cinerea* constitute a specialized extracellular defense and surface-maintenance system during mushroom morphogenesis.

## The nuclear proteome of *Schizophyllum commune*

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The dikaryotic life-stage of basidiomycetes is characterized by the true distribution of both different nuclei in the entire newly formed mycelium after mating of two compatible mating types. On genetic level, prerequisite for compatible mating is determined by the mating types loci, and subsequent induction of intracellular signaling. Phenotypically, a phase of nuclear migration followed by nuclear assortment in the tips of newly formed hyphae establish the dikaryon. However, it remains a mystery how the two different nuclei are distinguishing within a cell, if proteins are translated in the cytoplasm. It would be easier to accommodate differences independent of the mating type loci, if translation was directly performed in the nucleus without the requirement of targeting a protein from the cytosol specifically into one of the two nuclei. This consideration led us to address proteins enriched in the nucleus to potentially identify nuclear identity markers.

# Regulation of chitin synthesis and fruiting body development by the Chs4/Skt5 orthologs CSA-1 and CSA-2 in *Neurospora crassa*

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Chitin is a core structural component of fungal cell walls, which provides the rigidity and mechanical strength necessary to maintain cell plasticity and polarity. This structural homopolysaccharide is synthesized by chitin synthases (CHS), a conserved family of glucosyltransferase enzymes across the fungal kingdom. This study focuses on the chitin synthase activator proteins CSA-1 (NCU09322) and CSA-2 (NCU02592) in *Neurospora crassa*, orthologs of the *Saccharomyces cerevisiae* Chs4/Skt5 protein, which acts as a critical adaptor for Chs3 localization and activity during cytokinesis1,2. Analysis of the synteny of chs-4 and csa-1 revealed that both genes are highly conserved among Ascomycota species. Subcellular localization and dynamics of CSA-1 tagged with fluorescent proteins revealed its strong association with the tubular endoplasmic reticulum (ER), consistent with its SEL1-like repeat domains. Notably, changes in the subcellular localization of CHS-4-GFP and CHS-6-GFP in Δcsa-1/Δcsa-2 mutant strains suggest that both proteins can influence CHS transport to the apex. Additionally, both csa-1 and csa-2 have been demonstrated to be essential for the development of fertile perithecia and chitin synthesis. These findings highlight CSA-1 and CSA-2 as key factors in chitin synthesis, providing new insights into the molecular machinery governing cell wall biosynthesis in filamentous Ascomycetes.

1Trilla, J. A., Cos, T., Duran, A. and Roncero, C. (1997). Characterization of CHS4 (CAL2), a Gene of Saccharomyces cerevisiae involved in chitin biosynthesis and Allelic to SKT5 and CSD4. Yeast 13, 795–807.

2Oh, Y., Schreiter, J. H., Okada, H., Wloka, C., Okada, S., Yan, D., Duan, X. and Bi, E. (2017). Hof1 and Chs4 Interact via F-BAR Domain and Sel1-like Repeats to Control Extracellular Matrix Deposition during Cytokinesis. Current Biology 27, 2878-2886.e5.

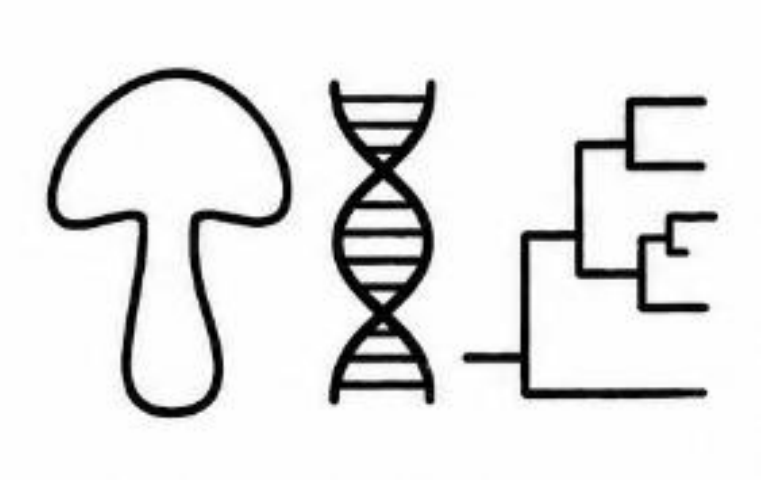
# A divergent Pho85 cyclin regulates polarized toxocyst morphogenesis in *Pleurotus ostreatus*

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Fungi can remodel their cell shape in response to environmental cues, allowing them to adapt to changing conditions and interact with other organisms. The oyster mushroom *Pleurotus ostreatus* paralyzes and kills nematodes using specialized lollipop-shaped structures called “toxocysts,” that form under nutrient-limiting conditions. The genetic control of the toxocyst development remains unclear. Using forward-genetic screens in P. ostreatus strain PC9, we identified multiple loss-of-toxicity (lot) mutants lacking toxocysts, revealing a strong correlation between toxocyst formation and nematicidal activity. Whole-genome sequencing and genetic mapping pinpointed the causative mutation in the lot1 mutant to PCL11, which encodes a previously uncharacterized Pho85 cyclin that is phylogenetically distinct from canonical Pho85 cyclins. Pcl11 physically interacts with the cyclin-dependent kinase Pho85, suggesting that the Pho85-Pcl11 complex regulates toxocyst development. We further found that the polarity regulator Cdc42, together with septins and F-actin, localizes to sites of toxocyst initiation and branching. Together, these findings suggest that Pcl11 controls polarized toxocyst formation by organizing conserved cell polarity and cytoskeletal machinery, providing new insight into how predatory fungi produce specialized structures for nematode killing.

# Genomics & Evolution



# A regulated mechanism for interspecific horizontal transfer of entire chromosomes in fungal pathogens

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Horizontal chromosome transfer (HCT) occurs in several fungal pathogens and typically involves accessory chromosomes; however, it remains unclear whether such transfers are regulated. Here, we demonstrate that a single accessory chromosome is transferred frequently and with remarkable specificity between different species within the fungal genus *Metarhizium*, both in vitro and in the field. In vitro, we observed highly specific transfers of only the accessory chromosome between different *Metarhizium* species, with up to 20% of asexual spores receiving the horizontally transferred chromosome. Furthermore, genomic analyses of 154 field isolates from around the world, representing 13 *Metarhizium* species, revealed more than 30 accessory chromosomes, the majority of which show signatures of past horizontal transfer. These mobile chromosomes share a core gene set enriched for predicted DNA-binding and chromatin-conformation functions. Preliminary data indicate that candidate genes within this core accessory chromosome gene set affect the transfer frequency of the accessory chromosomes. Collectively, these findings suggest the existence of a widespread, regulated mechanism of horizontal chromosome mobility in Metarhizium.

## Synthetic genomics to probe genome function: lessons from synthetic yeast and neochromosomes

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Synthetic biology is increasingly expanding from the design of individual genetic circuits to the construction and redesign of entire genomes. This shift creates powerful opportunities not only to engineer cells, but also to investigate fundamental biological functions through systematic genome-scale perturbation. This talk will discuss how synthetic genomics can serve as an experimental framework to study the relationship between genome architecture, chromosome organization, and cellular function. A central focus will be the Synthetic Yeast Genome Project, Sc2.0, the international effort to redesign and synthesize a complete eukaryotic genome. Beyond its technological ambition, Sc2.0 provides a programmable platform for biological discovery. Its redesigned chromosomes incorporate features such as genome streamlining, recoded elements, and inducible recombination sites, enabling controlled genome rearrangements and the systematic exploration of genotype–phenotype relationships. Particular emphasis will be placed on neochromosomes as synthetic genomic tools. By reorganizing genetic material outside its native chromosomal context, neochromosomes make it possible to test how gene order, copy number, regulatory environment, and chromosome structure influence biological activity. In this way, synthetic chromosomes become more than engineering achievements: they become experimental systems for uncovering context-dependent functions and hidden biological interactions. The talk will address how synthetic genomics can help reveal non-canonical, or “moonlighting,” roles of essential cellular machineries. As an example, the tRNA splicing endonuclease will be highlighted. This complex is classically known for removing introns from precursor tRNAs, but emerging evidence suggests broader functions beyond canonical tRNA processing. Neochromosome-based approaches and chromosome redesign offer a route to systematically investigate cellular functions on a molecular level.

# Genome expansion and phylogenetic history drive effector repertoire variation in *Helotiales*

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Introduction: Fungal secreted effectors are key mediators of plant–fungus interactions, but the relative roles of ecological lifestyle, phylogenetic history, and genome architecture in shaping effector repertoire evolution remain unclear. Methodology: Here, we investigated secreted effector gene repertoires across 90 species of *Helotiales r*epresenting Endophyte (n = 5), Freshwater saprobes (n = 32), Mycorrhiza (n = 9), and Phytopathogen (n = 44) lifestyles. Genomes were annotated, and screened for secreted effector candidates, followed by orthology-based identification of lineage-restricted orphan effectors. We then applied comparative genomic analyses and phylogenetically informed models to test whether effector abundance is better explained by ecological lifestyle or genome size. Results: Genome size differed among lifestyles, with mycorrhizal taxa exhibiting significantly larger genomes than freshwater, endophytic, and phytopathogenic species. Secreted effector gene counts also varied among lifestyles, with mycorrhizal fungi showing the highest average repertoires and phytopathogens the lowest, although some pathogenic taxa displayed marked effector expansions. Across *Helotiales*, genome size was positively associated with secreted effector abundance under phylogenetic generalized least squares regression, and strong phylogenetic signal indicated substantial evolutionary structuring of effector repertoire variation. Phylogenetically independent contrasts further supported coordinated evolutionary change between genome size and effector repertoire size, with sublinear scaling suggesting that effector expansion contributes to, but does not fully explain, genome enlargement. In contrast, orphan effector abundance was highly variable among species but not significantly structured by lifestyle, indicating predominantly lineage-specific patterns of effector innovation. Conclusion: our results suggest that secreted effector repertoire size in *Helotiales* is shaped primarily by genome architecture and phylogenetic history, with ecological lifestyle contributing secondary and lineage-specific effects. These findings highlight the evolutionary complexity of effector diversification across fungal lifestyles and suggest that freshwater fungi may retain molecular features associated with plant-associated interactions.

# Genomic background of Mycoparasitism in *Basidiomycota*

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*Pucciniomycotina*, one of three subphyla of *Basidiomycota*, spans an exceptional ecological diversity, ranging from plant pathogens (including rusts and anther-smuts) through insect pathogens, to a taxonomically scattered array of mycoparasites—fungi that parasitise other fungi. Compared to *Ascomycota*, where mycoparasites are mostly concentrated in the order Hypocreales, mycoparasites in Basidiomycota are distributed over all subphyla. In *Pucciniomycotina* alone, the mycoparasitic lifestyle arose in at least seven out of 10 currently recognised classes, and at least three major cellular interaction mechanisms were documented. However, cultures and molecular sequence data are unavailable for the majority of these mycoparasites. Consequently, most of our current insights into the genomic basis of mycoparasitism come from some well-studied examples in *Trichoderma* (*Hypocreales*). But how is the mycoparasitic lifestyle characterised at the genomic level in *Pucciniomycotina*? And how do these mycoparasites differ from closely related plant pathogens? Addressing these questions requires dedicated sampling of mycoparasite genomes, as well as insight into the evolutionary relationships within this subphylum. Previous multilocus approaches have failed to resolve relationships among the subphylum's classes. We present the first dedicated phylogenomic study of *Pucciniomycotina* together with a comparative-genomic characterisation of its mycoparasites. We newly sequenced genomes of seven mycoparasitic *Pucciniomycotina* using the ONT platform, covering all but one of the classes in which mycoparasitism is documented. An additional 70 genome assemblies were obtained from GenBank and JGI. All genomes were decontaminated, repeat-masked and annotated de novo. We evaluated phylogenomic relationships within *Pucciniomycotina* based on coalescent and network approaches to understand diversifications at the deep nodes of the subphylum. Moreover, we examined genomic signatures of the mycoparasitic lifestyle, by profiling their effectors, CAZymes, and biosynthetic gene clusters. This work delivers a phylogenomic backbone for *Pucciniomycotina* and an integrated view of its mycoparasites, setting the stage to test how often, and through which genomic innovations, mycoparasitism evolved.

# Not all chromosomes are equal: chromosome-specific aneuploidy stability in *Candida albicans*

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*Candida albicans* exhibits high genome plasticity, with aneuploidy - an imbalance in chromosome copy numbers - being well tolerated. Aneuploidy has been linked to the emergence of drug evasion mechanisms like resistance and tolerance, but the dynamics and mechanisms of aneuploidy adaptation remain poorly understood. Here, we leveraged a collection of 200 natural *C. albicans* strains isolated from diverse clinical and environmental sources, each containing whole-chromosome copy number alterations, to study aneuploidy stability. We propagated isolates under non-stressed conditions for 150 generations and profiled both fitness and proteomes of evolved and ancestral strains using high-throughput methods. Using proteomic data, we inferred relative chromosome copy numbers of each isolate by exploiting the proportional scaling of protein abundance with chromosomal dosage, thus tracking karyotype stability across clades. Surprisingly, we find that in ~20% of aneuploid strains, aneuploidy can be stably maintained. Rare aneuploidies (e.g. Chr6, Chr7) often persist, whereas more common ones (e.g. Chr4) tend to be unstable and revert to euploidy, suggesting chromosome-specific cellular constraints on the ability of cells to tolerate aneuploidy. Furthermore, we observe that stable aneuploids exhibit significantly increased minimum inhibitory concentrations against multiple classes of antifungals. Global proteome comparisons reveal adaptations to aneuploidy, with stable isolates upregulating cell wall components and amino acid metabolism, whereas reverting isolates show higher translational activity. We also observe increased fitness of stable aneuploids in response to caffeine exposure, consistent with a role of cell wall integrity in the maintenance of aneuploidy and, potentially, associated resistance to antifungals. Together, these results reveal chromosome-specific patterns of aneuploidy stability across diverse genetic backgrounds and link stable aneuploidy to proteome remodeling and reduced antifungal susceptibility.

# Whole-Genome Sequencing of *Aspergillus oryzae*: Identifying putative enzymes involved in Aflatoxin B1 degradation

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In this study, the genome of *Aspergillus oryzae* MRI389, a fungus widely used in industrial fermentation, was sequenced using Illumina platforms for short-read sequencing and Oxford Nanopore Technologies for long-read sequencing. As part of the world nutrition project SoLFOOD, *A. oryzae* MRI389 is being investigated as a potential biological tool for the detoxification of maize contaminated with aflatoxin $B_1$.

After confirming aflatoxin $B_1$ degradation in LB broth through high-performance liquid chromatography (HPLC), genome-wide sequence analysis enabled the identification of putative aflatoxin B1-degrading enzymes, leading to the selection of nine hypothetical proteins as candidate enzymes. Many of the aflatoxin $B_1$-degrading enzymes described in previous studies are classified as oxidoreductases (E1), whereas eight multi-copper oxidases and one aflatoxin $B_1$ aldehyde reductase have been identified in the *A. oryzae* genome. The next step will be to evaluate their potential involvement in aflatoxin $B_1$ degradation through gene expression analysis using droplet digital PCR (ddPCR).

Access to genome sequences is essential for the rapid and accurate identification of fungi, as morphological characteristics in the immense diversity of the fungal kingdom are often insufficient to reliably determine their ecological function and impact on ecosystems [1]. Whole-genome sequencing provides thereby a comprehensive catalogue of an organism's genetic repertoire and is a crucial prerequisite for identifying genes and metabolic pathways involved in specific biotechnological processes.

# Comparative Genomics and Evolution of the Sexual Reproduction Mode Across the Genus *Sordaria*

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Sexual reproduction promotes genetic recombination and enhances more adaptive progeny in fungi. In *Ascomycota*, the sex determinants are encoded by the mating-type (MAT) locus, which are the mat A (MAT1-1) and mat a (MAT1-2) idiomorphs. The two primary modes of reproduction are homothallism and heterothallism. The model organism *Sordaria macrospora* is homothallic, carrying both idiomorphs in a single genome, whereas heterothallic species typically harbor only one idiomorph. Homothallic *Sordaria* species have not been reported to produce asexual spores. This study investigates the evolutionary development of sexual and asexual reproduction in the genus *Sordaria* by integrating newly generated genomes from homothallic species, *S. fimicola* and the heterothallic species, *S. brevicollis* mat A; *S. sclerogenia* mat A and mat a with publicly available genomes. Using long-read de novo assemblies, we confirm that *Sordaria* genomes contain seven chromosomes, with the MAT locus consistently located on the first chromosome. Comparative analyses reveal that *S. brevicollis* possesses a larger genome with reduced GC content, while *S. sclerogenia* resembles homothallic species in both genome size and GC composition. Furthermore, genome-wide dN/dS analyses showed that homothallic species exhibit higher values than heterothallic species, suggesting that homothallic lineages experience relaxed purifying selection and accelerated evolution across the genome. We also identified a substantial contribution of transposable elements (TEs) to genome plasticity. Remarkably, in heterothallic species, GC content strongly correlates with gene density and TE distribution, indicating that active TEs may shape genomic divergence between heterothallic and homothallic *Sordaria* lineages. Additionally, divergence analysis based on transposon insertion ages calculated by Kimura distances indicated that TE proliferations are older in heterothallic species than in homothallic species. Overall, this research provides new insights into the genomic and evolutionary factors driving reproductive diversity within the genus *Sordaria*.

# Genome dynamics and gene repertoire variation in the nematode-trapping fungus *Arthrobotrys oligospora*

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Fungi are globally distributed and rapidly adapt to changing environments. *Arthrobotrys oligospora* is an orbiliaceous nematode-trapping fungus widely used as a model for fungal predation. However, little is known about its intraspecific genetic diversity or how its genome evolves across environments with distinct pathogens, competitors, and prey. Here, we sampled 14 A. *oligospora* strains from locations across Taiwan and generated telomere-to-telomere genome assemblies for each strain. Comparative analyses revealed extensive structural variation among strains, including large-fragment insertions, deletions, duplications, and translocations. The cumulative length of structural variants reached up to ~15% of the genome size. We next assessed whether genomic variation contributed to gene repertoire differences. Across all strains, we identified 14,558 orthologous groups, of which 73% were shared by all strains and defined as core genes, whereas 27% were subjected to frequent gain or loss and defined as accessory genes. Functional annotation showed that accessory genes were enriched in functions associated with environmental interactions, including fungal immunity, self recognition, and secondary metabolism such as lipid and polyketide biosynthesis. Together, these results suggest that *A. oligospora* genomes are highly dynamic, facilitating gene repertoire diversification that may shape adaptation to diverse nematode prey and changing ecological environments.

# Signal Reliability and Nutritional Rewards Drive Divergent Evolutionary Trajectories in the Nematode-Trapping Fungus *Arthrobotrys oligospora*

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Nematode-trapping fungus *Arthrobotrys oligospora* captures nematodes to meet nitrogen requirements. Facultatively predatory, it grows saprophytically but forms adhesive traps upon sensing low nitrogen and prey signals. This inducible strategy purportedly conserves metabolic resources. However, the evolutionary trade-offs balancing conservation and sensory thresholds remain underexplored. To investigate how signal reliability and nutritional reward influence the evolution of this predatory lifestyle switch, we employed a long-term experimental evolution approach. Here, we report results from 36 replicate populations evolved under three ecological conditions: (1) Starvation Control (SC; no prey and no prey signal); (2) Predation-Reward (PR; continuous access to live prey, so prey signals reliably predict nutritional reward); and (3) Interrupted-Reward (IR; prey-associated signals are present, but prey is removed before capture and digestion). We observed high phenotypic divergence across the evolved lineages. First, evolved populations diverged in ascaroside-induced trap morphogenesis, indicating that prey-pheromone responsiveness can evolve under different ecological conditions. Second, some lineages showed increased spontaneous trap formation in the absence of prey cues, suggesting that evolution can shift the activation threshold for trap morphogenesis. Third, when predatory investment was compared with reproductive output, lineages that produced more traps tended to produce fewer conidial clusters, suggesting a potential trade-off between predation and asexual reproduction. At the later evolutionary time point, generation 74, this predation-biased pattern became more apparent, with more lineages showing stronger spontaneous trap-associated phenotypes. Among the evolved populations, one lineage, PR5, stood out as an especially extreme example: it showed strongly elevated trap production, including both spontaneous and nematode-induced trap formation, together with reduced conidiation and reduced vegetative growth. We therefore interpret PR5 as one extreme predation-biased lineage, rather than as the average outcome of the whole condition. Together, these findings suggest that the regulation of fungal predation is evolvable: prey-pheromone responsiveness, the activation threshold for trap morphogenesis, and allocation between predation, reproduction, and growth can all shift in response to ecological conditions.

# Diversity and Functional Relevance of the Fungal Microbiome in the Model Organism Hydra vulgaris AEP

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The freshwater polyp *Hydra vulgaris* AEP is a key model organism in metaorganism research due to its low complexity, ease of experimental manipulation and well-defined bacterial microbiota. While the importance of its bacterial microbiome for the host development, health, and contractile behaviour is well established, its fungal microbiome remains largely unexplored. The *Hydra* microbiome is shaped not only by complex interactions with the host and the environment, but also by interactions among associated microorganisms. Therefore, identifying the fungal taxa present and understanding their functional roles are essential for a more complete understanding of the metaorganism. To investigate the fungal microbiota associated with Hydra, we established a fungal culture collection from both laboratory-maintained polyps and individuals maintained in natural lake environments. Penicillium strains were consistently isolated from both habitats, suggesting a stable association with *Hydra*. To assess potential interactions between fungi and bacterial community members, we tested fungal isolates against the most common bacterial members of the Hydra microbiome, using in vitro plate-based assays. Of the 110 combinations tested, 20 showed interactions, predominantly involving fungal inhibition of bacterial growth, with the different *Penicillium* isolates exhibiting varying inhibition patterns. Finally, whole-genome sequencing of the *Penicillium* isolates, identified as *P. brevicompactum and P. olsonii*, revealed predicted secondary metabolite biosynthetic gene clusters, including several associated with antibacterial compounds that may provide a genomic basis for the inhibitory activities observed in vitro. Together, these results provide the first genomic and functional characterization of Hydra-associated fungi and offer new insights into their potential role of fungi in shaping microbial interactions and community dynamics within the host-associated microbiota.

# Whole-genome assessment of interspecific hybridization, evolutionary history, and virulence in opportunistic fungi

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Understanding the evolutionary history and life strategies of filamentous fungi is often complicated by ambiguous taxonomy and complex genomic phenomena. Interspecific hybridization, in particular, remains a relatively understudied phenomenon, despite increasing evidence that hybrid genomes significantly impact ecological adaptation, human health, and evolutionary innovation. In microscopic fungi, the identification of hybrids is challenging, as phenotypic traits overlap with parental species, leading to potential misidentification in clinical settings. Nevertheless, several interspecific hybrids have been reported as causative agents of opportunistic infections, highlighting their medical relevance.

In this study, we investigate interspecific hybridization within the genus *Aspergillus*, focusing on series *Viridinutantes*, a group that includes opportunistic pathogens. Under in vitro conditions, these species form hybrids, providing a model system to assess hybrid genomes and their impact on phenotypic and functional traits. We generated whole-genome sequences of parental and hybrid strains using long-read Oxford Nanopore technology, enabling the reconstruction of highly contiguous assemblies.

Our analyses reveal that these hybrids are allodiploid, containing genomic contributions from both parental species, but frequently exhibit varying degrees of aneuploidy. Morphologically, the hybrid strains are distinct from parental lineages, indicating substantial phenotypic divergence. To evaluate the potential impact on health and pathogenic traits, we assessed virulence using the *Galleria mellonella* infection model. Notably, *Aspergillus felis* and the generated *A. felis × A. udagawae* hybrid exhibited virulence comparable to that of clinical *A. fumigatus* isolates. This demonstrates that tested hybrid strains do not show reduced virulence compared to their parental lineages or established major pathogens.

Through interspecific hybridization, opportunistic fungi can generate genomically and phenotypically distinct lineages without a loss of virulence. This underscores the necessity of integrating whole-genome assessments to understand the evolution and taxonomy of elusive fungal pathogens with clinical relevance.

# Revisiting the taxonomy of genus *Aspergillus* series *Viridinutantes*: Genome-based species delimitation accounting for complex species boundaries

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Genus *Aspergillus* series *Viridinutantes* is a group of ascomycete fungi closely related to *Aspergillus fumigatus*, an opportunistic fungal pathogen recognized as critically important by the World Health Organization. Likewise, the series *Viridinutantes* includes several species frequently recovered from clinical material. Many of the *Viridinutantes* species have been recognized over the last few decades because of their cryptic nature – the most recent being *A. bezerrae*, described in 2019. The latest taxonomic revision of this group was based on a multilocus approach (four genes) and revealed conflicting signals for some species – particularly *A. udagawae* and *A. felis*. Crossing experiments also demonstrated the ability of these species to form viable interspecific hybrids. We hypothesize that whole-genome data can successfully resolve the taxonomic conflicts within this series. To test this, we compared different scales of genomic information, ranging from core conserved genes to genome-wide polymorphism data.

In this study, we analysed 88 genomes representing *A. bezerrae, A. wyomingensis, A. udagawae, A. felis*, and *A. pseudoviridinutans*. Of these, 83 were sequenced de novo on the Illumina platform, while the remaining genomes were obtained from the NCBI GenBank database. To evaluate the resolution of different genomic data subsets, we utilized three approaches: (1) 55 universal fungal core genes (UFCG) analysed via single-gene species delimitation methods (ABGD, GMYC, bGMYC, PTP, and mPTP); (2) orthologous genes from OrthoFinder analysed with ABGD and Tr2; (3) single nucleotide polymorphisms (SNPs) evaluated using population structure analysis. The resulting species hypotheses are further integrated and validated using morphological and other diagnostically significant traits.

Our findings demonstrate the potential of genome-scale data, combined with rigorous data selection and complementary species delimitation approaches, to improve taxonomic resolution. This framework provides a robust basis for resolving complex species boundaries and may be applicable to other taxonomically challenging fungal groups.

# Hidden diversity and ecological adaptation in the genus *Pseudogymnoascus*

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*Pseudogymnoascus* is a diverse genus of predominantly cold-adapted fungi occupying a wide range of environmental and host-associated niches, including soils, cave systems, and vertebrate hosts. Despite increasing interest in the genus driven by the emergence of the bat pathogen Pseudogymnoascus destructans, its overall diversity and adaptive strategies remain poorly understood.

To explore evolutionary relationships and ecological diversification within Pseudogymnoascus, we analyzed approximately 200 isolates originating from cave systems, rodents, reptiles, clinical material, and other environmental sources. Multilocus phylogenetic analyses based on ITS, RPB2, MCM7, and TEF markers revealed substantial hidden diversity, including several previously unrecognized lineages and putative novel taxa.

Preliminary physiological characterization indicates marked differences among lineages in temperature and salt tolerance. While some taxa display increased thermotolerance, others exhibit narrow thermal niches consistent with adaptation to cooler environments. Several lineages also appear to show strong associations with specific hosts, particularly rodents.

Ongoing comparative analyses integrating phylogenetic, physiological, genomic, and metabolomic data are beginning to reveal patterns of ecological specialization and secondary metabolite diversification within the genus. Current results indicate that Pseudogymnoascus is not restricted to a narrowly defined cold-adapted lifestyle but comprises lineages differing in environmental preferences, thermal tolerance, and host associations.

# European *Puccinia tritici f.sp. striiformis* races – a genomics approach to stripe rust of wheat

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The global expansion of wheat stripe rust due to the intercontinental spread of new lineages of the causative pathogen *Puccinia striiformis f. sp. triti*ci (Pst) has driven large changes in the epidemiology of the disease over the last two decades. A rise in epidemics through the spread of temperature tolerant, more aggressive lineages and by introducing virulences that defeat previously effective host resistance rendered stripe rust an increasingly abundant and severe disease and a significant threat to cereal production worldwide, including regions where the disease previously has been minor. Incursions of new lineages invoked significant shifts in the European population that likely contributed to a change in the virulence spectrum and adaptive potential in the population, which likely influenced the trajectory of future outbreaks and breeding priorities in this region.

# Unraveling the Pathogenicity of the *Candida parapsilosis* Species Complex through Comparative Pan-Genomic Analysis

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Invasive fungal infections pose a life-threatening risk to millions of individuals each year. The *Candida parapsilosis* species complex (CPSC) is a major cause of human fungal disease and comprises three major species: *C. parapsilosis* (Cpara), *C. orthopsilosis* (Cortho), and *C. metapsilosis* (Cmeta). Despite their close phylogenetic relationship, Cpara accounts for over 90% of infectious cases within this complex. Genomic plasticity is generally regarded as a key driver of adaptation to different environments, yet Cpara's clinical predominance challenges this assumption given that its genome exhibits the lowest levels of heterozygosity within the CPSC. This suggests that alternative genomic strategy may underlie Cpara's success as a pathogen. To identify the evolutionary genomic features underpinning Cpara's clinical advantage, we conducted a comparative genomics study spanning 1,051 CPSC genomes sampled across North America, Europe, and Asia. Analysis of population structure and linkage disequilibrium decay showed evidence of frequent recombination in Cpara, which contradicts its long-standing characterization as a predominantly clonally reproducing species. Notably, however, the recombination does not translate into substantial genomic diversity. Demographic modeling suggests that Cpara underwent a more recent bottleneck followed by a rapid population size expansion compared to Cortho and Cmeta, but that despite the ongoing recombination in Cpara, its genomic diversity is still significantly lower than its closely related species. The current findings suggest that genomic streamlining driven by a historical population bottleneck may have contributed to Cpara's successful adaptation during infection, potentially explaining its higher prevalence in clinic compared to its two sister species.

# Genome reshuffling as a route to specialization? Chromosome-level insights from the bark beetle symbiont genus *Geosmithia*

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Structural genome evolution is increasingly recognized as a major driver of fungal adaptation, yet current knowledge remains strongly biased toward plant pathogens. Here, we establish the bark beetle–associated fungal genus *Geosmithia* as a model for studying genome evolution across ecological transitions in predominantly non-pathogenic fungi. *Geosmithia* species occupy diverse ecological niches, ranging from generalists associated with multiple host trees and beetle vectors to conifer specialists and obligate ambrosial nutritional symbionts cultivated by a specific ambrosia beetle. Using chromosome-level genome assemblies from eleven species representing independently evolved ecological strategies, we investigated how genome architecture, repetitive DNA, and gene family evolution contribute to ecological diversification.

Comparative genomics revealed extensive intra- and interchromosomal rearrangements across the genus, including among closely related species with nearly identical nucleotide identity, suggesting that rapid chromosome restructuring can accumulate over short evolutionary timescales. Breakpoints frequently occurred within gene-rich regions, indicating that genome evolution in *Geosmithia* is likely driven by mechanisms other than transposable element expansion.

Hi-C analyses revealed typical Rabl chromosome organization, but unusual centromere structure characterized by low repeat content and highly conserved syntenic gene blocks, indicating stable centromeric positions despite extensive genome reshuffling. Our data further suggest that centromere identity may be determined by a highly divergent CenH3 variant protein, although this requires experimental validation.

Despite similar genome sizes across species, specialists underwent extensive gene losses and display higher proportion of repetitive sequences in their genomes. Gene family contractions in specialists were identified across multiple functional categories, which is consistent with metabolic streamlining accompanying ecological specialization.

Our results demonstrate that extensive structural genome evolution can occur in gene dense fungal genomes and establish *Geosmithia* as a powerful system for studying the interplay between chromosome evolution and ecological diversification.

# Improving the systematics of *Hypoxylaceae* by using comparative genomics

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The family *Hypoxylaceae* (*Xylariales*) comprises cosmopolitan fungi with endophytic, saprobic and opportunistic phytopathogenic life stages often forming conspicuous, carbonized stroma on plant tissue in which their perithecia are embedded. Many taxa are described to store large amounts of pigments in the stroma, which has attracted considerable interest during screening campaigns on the search of bioactive compounds. In tandem with phylogenetic and morphological data, compound presence/absence patterns proved useful fingerprinting for chemotaxonomic considerations in polyphasic scenarios. Recently, a pilot study sequencing and analyzing the first 13 genomes of the main hypoxylaceous lineages confirmed the current systematic concept. However, *Hypoxylon*, the most species-rich genus in the family, remains paraphyletic in recurrent state-of-the-art multi-locus phylogenies, heralding further rearrangements once more data becomes available. Here, we embark on this issue by analyzing a total of 95 publicly available *Hypoxylaceae*-derived genomes in a comparative genomic framework. A phylogenomic species tree was calculated following a coalescence-based approach from 1695 BUSCO genes. Gene and site-concordance factors were calculated from single gene trees and mapped against the species tree to estimate phylogenetic conflict. Genome synteny was analyzed in an all-vs-all approach to map gradual changes in genome architecture throughout the species tree. Here, we found that the clades resolving in different genera form macrosyntenic clusters, corroborated with longer syntenic blocks, while different lineages share micro- and mesosynteny throughout the family independent of their phylogenetic placement. This approach provides new insights about species delimitation within *Hypoxylaceae*. In particular, the *H. rubiginosum* complex was shown to form a clear, self-syntenic cluster congruent with its placement in the species tree, demonstrating evolutionary independence from other hypoxylaceous genera, which we hence propose to accommodate with a new genus. Other selected taxa showing mixed phenotypic characters are discussed in the context of phylogenetic reconciliation frameworks estimating gene flow. Summarizing, this study not only improves the systematics of the *Hypoxylaceae*, but also demonstrates the importance of assessing phylogenetic conflict in the era of genome sequencing and emphasizes on the opportunities this approach offers.

# Propagation of [D1,2]-type spliceosomal twin introns in *Xylariales* taxa

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Spliceosomal U2 introns are pervasive in nuclear transcriptomes but their continuous propagation, hence, their persistence or amplification in most contemporary Eukaryotic lines, remains a vexing question. The non-coding intronic sequences alternating with coding exons in pre-mRNAs do not necessarily consist of one canonical U2 intron. Complex intronic sequences called [D1,2] stwintrons consist of nested U2 introns, where an internal intron splits the 5'-donor of an external intron between its first and second nucleotide (nt).

Almost all stwintrons described to date are of the [D1,2] type, suggesting unique means for their duplication. Sequence-similar [D1,2] stwintrons are typically integrated at new intron positions, specific for one species. Two hundred eighty-eight sequence-similar [D1,2] stwintrons were identified in the genomes of 14 *Xylariales*. Occasional missplicing was apparent, where almost the entire stwintron was excised as one canonical intron. Near-identical sister stwintrons were identified in *Xylaria sp*. MSU SB201401 and *Xylaria* longipes that share near-terminal inverted repeat elements of 10-nt length named 5'-NTIRE-10 and 3'-NTIRE-10, which are complementary as RNA. Complementary NTIRE-10 partners were also present in three *Hypoxylaceae* species. These NTIRE-10s can form a near-terminal double-stranded RNA stem structure that brings in close proximity the terminal G's of the [D1,2] stwintron and of its alternative misspliced intron. The exact folding of the interior stwintron RNA appears irrelevant. Ten of the stwintrons with complementary NTIRE-10s are present in *Xylaria* sp. MSU SB201401, implying that [D1,2] stwintron duplication occurs frequently in this species.

# A New Backbone Phylogeny for *Ustilaginales* (Smut Fungi) Helps Understand Host-Parasite Interaction

Markus Haferkamp, Sean Blundell, Nils Hassel, Qi-Ming Wang, Martin Kemler, Dominik Begerow

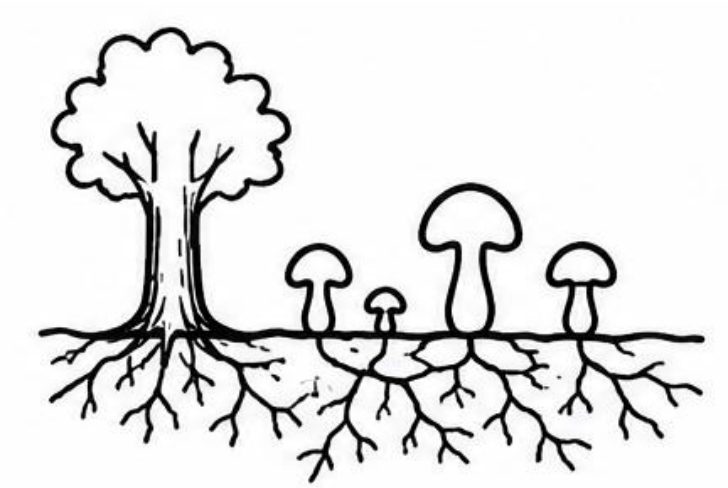
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Smut fungi (*Ustilaginales*) are a monophyletic group of biotrophic plant-parasitic fungi that are characterised by their 'smutty', blackish teliospores. Most *Ustilaginales* are parasites of grasses, including on important food crops such as maize, oat, sorghum, sugarcane, and wheat.

The majority of smut fungi are highly host specific, with some species exhibiting a one-to-one association with a single host species, making them an attractive model system to study host-parasite co-evolution and the molecular mechanisms of host specificity. Previous experiments in the model species *Mycosarcoma maydis* and *Sporisorium reilianum* have demonstrated that traits governing host-fungus interactions (i.e., host defences and host development) involve a set of small secreted fungal molecules known as effectors. However, linking the evolution of specific effectors to host specificity remains challenging due to insufficient evolutionary resolution across the *Ustilaginales*. To overcome this limitation we leveraged genomic data from a broad sampling of fifty species within *Anthracoideaceae* and *Ustilaginaceae*. Using this new dataset we present an updated, fully resolved phylogeny for the *Ustilaginales*. Preliminary analyses of effector evolution confirm established functional patterns: all studied species contain a highly-conserved, analogous 'core' effectome that could be crucial for successful infection, alongside a divergent 'accessory' effectome that undergoes rapid evolution and frequent gene duplication.

The solid phylogenetic framework built in this study not only resolves evolutionary patterns, but also provides the foundation to understand the mechanisms that underlie the observed patterns of high conservation and high plasticity in effector evolution of *Ustilaginales*. We hope to use this deepened theoretical understanding to identify new species- and host-specific effector candidates that are crucial to pathogen evolution and successful host infection.

# Ecosystem Functions & Communities



## Field-Scale Benefits of Mycorrhizal Fungi in Agroforestry

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Mycorrhizal fungi are known to enhance plant nutrition, growth, and health, yet most evidence has focused on arbuscular mycorrhizal associations under controlled laboratories or greenhouse conditions. In contrast, the role and practical application of fungal inoculant in field-scale agricultural systems, particularly those involving ectomycorrhizal fungi like agroforestry systems, remain insufficiently studied. In this study, commercially available inoculants containing mixtures of both ecto- and endomycorrhizal fungi were applied during the establishment of an agroforestry (alley cropping) system consisting of poplar clones (*P. nigra x P. maximowizcii*), red currant shrubs (*R. rubrum* cv. Jonkheer van Tets), and annual crop rotations of summer barley, faba bean and maize.

Successful establishment of mycorrhizal associations was verified through quantification of root colonization across all plant groups. We then evaluated the functional effects of fungal inoculation on crop yield, tree growth, and plant health under field conditions. Mycorrhizal fungi significantly improved both crop and wood yields beginning in the second year after planting. In crops, increased yield was primarily associated with higher thousand-grain weight rather than enhanced vegetative growth, whereas inoculated poplar trees showed greater secondary growth. Furthermore, we found inoculated trees to be less susceptible to poplar beetle infestation, exhibiting reduced feeding damage and lower mortality compared with non-inoculated controls.

Our findings demonstrate that beneficial plant–fungus interactions established through commercial mycorrhizal inoculation can be successfully translated from controlled environments to agroforestry field systems. In particular, ectomycorrhizal fungi emerged as promising biostimulants for improving productivity and resilience in agricultural practice.

# Functional Diversity of Yeasts Shapes Communities and Ecosystem Processes

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Yeasts have traditionally been regarded as fast-growing saprobes specialized in exploiting readily available simple sugars. However, phylogenetic and ecological studies have fundamentally revised this view, revealing that many yeast lineages are closely related to parasites of plants and fungi, and possess adaptations that enable survival across a broad range of natural environments. Rather than being confined to sugar-rich substrates, many yeasts thrive under oligotrophic conditions and tolerate multiple environmental stresses, including low temperatures, desiccation, and nutrient limitation. Our work explores the functional diversity of yeasts inhabiting plant surfaces, floral nectar, tree fluxes, and soils. These yeasts exhibit remarkable metabolic versatility, including the utilization of diverse carbon sources, efficient nitrogen scavenging, and the accumulation of storage compounds that promote persistence under fluctuating environmental conditions. Such traits contribute not only to individual fitness but also to the establishment and maintenance of yeast communities across contrasting habitats. Beyond their physiological adaptations, yeasts actively influence ecosystem processes through the production and modification of biologically active metabolites. We have shown that nectar yeasts produce a diverse array of metabolites from only three major nectar sugars. Phyllosphere yeasts synthesize plant growth hormones, highlighting their capacity to influence host physiology, while volatile organic compounds produced by yeasts associated with plant surfaces, fruits, nectar, and insects mediate interactions with plants, pollinators, and other microorganisms. Despite their considerable phylogenetic diversity, these yeasts share common chemical signatures, suggesting convergent evolution of metabolite-mediated ecological functions.Our findings demonstrate that functional diversity is a key driver of yeast ecology. Metabolic flexibility, stress tolerance, and chemical communication enable yeasts to occupy diverse ecological niches, while physiological diversification among closely related species promotes niche partitioning, community assembly, and ecosystem functioning in plant-associated environments. Understanding these functions provides a mechanistic framework for explaining the remarkable ecological success and diversity of yeasts.

# FUNACTION: Aquatic Fungal Biodiversity Across Europe Within The Scope Of Conservation and Monitoring

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Fungi comprise taxonomically and evolutionary diverse taxa and are ubiquitous in aquatic ecosystems worldwide, including many aquatic lineages. As saprotrophs, parasites, and symbionts they play essential roles in cycling of matter and nutrients across the aquatic landscape, ensuring ecosystem health, and stability. However, their biodiversity and functional roles in the aquatic realm remain elusive, and they are therefore excluded from mainstream conservation and monitoring efforts. A multinational initiative, FUNACTION, address these gaps. FUNACTION tests the efficacy of existing nature protection sites for aquatic fungi and builds the necessary knowledge, tools, and networks for conservation and monitoring of this elusive group. By utilizing Third-Generation sequencing approaches, rich metadata, and pan-European sampling with centralized analysis, FUNACTION relates fungal diversity to environmental and anthropogenic drivers. Characterization of fungal diversity across watersheds, from above the Arctic Circle, to northern Portugal, and across a year, as well as a multinational coordinated leaf litter decomposition study have now been completed. A 'hot off the press' view on AF diversity within Europe will be presented. By this collaborative effort, we are generating critical knowledge on fungal diversity in aquatic ecosystems by utilizing advanced molecular techniques and data integration, ultimately aiming to include fungi in routine monitoring programs and develop effective conservation strategies for these vital, yet overlooked, components of aquatic ecosystems.

# The hydrolysis of sucrose by *Zymoseptoria tritici's* secreted invertase impacts the antagonism of leaf-associated bacteria

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*Zymoseptoria tritici* is a highly specialized hemibiotrophic leaf pathogen that causes Septoria Tritici Blotch (STB) in wild and domesticated wheat. This pathogen secretes an array of molecules in the leaf apoplast that manipulate the immunity and metabolism of the host. Moreover, some of these effectors show antimicrobial activity in vitro and might be relevant for the competition against the host microbiome, as demonstrated in other fungal pathogens. Our previous work revealed that *Z. tritici* has the ability to enhance the growth of leaf- associated bacteria. This activity is linked to the secretion of an invertase that hydrolyzes sucrose around the fungal hyphae. While fungal invertases are important for carbon uptake, it has been suggested that fungal-derived hydrolysis products can also be relevant in the context of microbial interactions. Since carbon compounds are important to the production of bacterial antimicrobial molecules, we hypothesize that the pathogen modifies carbon availability in the colonized host tissue to reduce antifungal activity, and thereby antagonism, of cooccurring bacteria.We aimed to test this hypothesis and assess the role of the secreted *Z. tritici`s* invertase (SUC3) in pathogen-host and pathogen-microbiome interactions in a pathosystem including *Z. tritici* Zt469, *Aegilops cylindrica*, and leaf-associated Pseudomonas. Using SUC3 deletion mutants and heterologous expression of the invertase, we confirmed that the hydrolysis of sucrose enhances the growth of antagonistic Pseudomonas. The antifungal activity of Pseudomonas is reduced when co-cultured with Z. tritici in a glucose medium, suggesting that the invertase-derived monosaccharides reduce bacterial antagonism against *Z. tritici*. This is further evidenced by the fact that antifungal activity is higher in sucrose when co-cultured with the SUC3 deletion mutants compared to wild-type *Z. tritici*. Preliminary in vivo assays demonstrate that SUC3 impacts the pathogen's virulence both in gnotobiotic plants or plants inoculated with a Pseudomonas synthetic community. Our work shed new light on the mechanism of interaction between pathogens and the host microbiome that could be exploited to improve future biological control strategies.

# Host Health is influenced by the Foliar Mycobiome of Giant Sequoia During Decline

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Giant Sequoia (*Sequoiadendron giganteum*) is increasingly affected by decline symptoms, such as tip blight and dieback of entire tree crowns, across its European introduced range. However, little is known about the fungal communities associated with healthy and declining trees. In this study, foliar samples from 40 Giant Sequoia trees (20 asymptomatic and 20 symptomatic) in Freiburg, Germany, were analysed using high-throughput ITS amplicon sequencing combined with culture-based isolation methods to investigate how fungal community composition and interaction patterns vary with host health status. A total of 5,295 fungal ASVs were identified, with fungal communities dominated by Ascomycota and Basidiomycota and differing significantly between asymptomatic and symptomatic trees. Symptomatic trees were enriched in genera containing important opportunistic fungi, particularly *Botryosphaeria,* while *Neofusicoccum* was recovered exclusively from symptomatic trees by culture. In contrast, asymptomatic trees were enriched in genera such as Peltaster, which occurred only at very low abundance in symptomatic samples. In addition to changes in community composition, fungal co-occurrence patterns also differed between asymptomatic and symptomatic trees. Asymptomatic trees supported a single, highly connected association network, whereas symptomatic trees exhibited a fragmented network with four distinct modules. Furthermore, *Botryosphaeria* and *Neofusicoccum* displayed contrasting co-occurrence patterns between asymptomatic and symptomatic trees, suggesting that associations involving these taxa differ according to host health status. These patterns indicate that declining health in Giant Sequoia reflects a broader restructuring of mycobiome organisation rather than a single dominant pathogen. Our study provides the first characterization of the Giant Sequoia foliar mycobiome and establishes a baseline for future studies on fungal community dynamics and disease monitoring in this species across its European introduced range.

## Yeast Community Assembly in Sunflower Nectar

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Floral nectar is a highly selective microbial habitat characterized by high sugar concentrations, limited nutrient availability, and transient microbial dispersal. Despite these challenging conditions, specialized yeasts successfully colonize nectar and form distinct communities. We investigated the assembly and functional traits of yeast communities inhabiting sunflower (*Helianthus annuus*) nectar by combining field and laboratory experiments.

A pollinator-exclusion experiment was conducted on sunflowers using five treatments: access by day-active pollinators, night-active pollinators, both, or none, as well as controlled pollination by Bombus terrestris. Nectar, air, and bumble bee gut samples were analyzed using cultivation-based methods combined with MALDI-TOF MS and DNA barcoding. We also performed growth assays across different sugar concentrations and tested the tolerance of selected strains to two fungicides.

Nectar yeast communities were species-poor and strongly dominated by the nectar specialist *Metschnikowia reukaufii* in flowers visited exclusively by day-active pollinators. In contrast, flowers exposed only to night-active pollinators or fully excluded from insect visitation contained few or no yeasts. The absence of *M. reukaufii* from air samples suggests that bees are the primary vectors introducing this yeast into sunflower nectar. Fungal communities in the guts of captive bumble bees varied with diet, indicating a potential link between floral yeast availability and gut mycobiome composition. Furthermore, nectar-specialist yeasts exhibited high tolerance to elevated sugar concentrations and toxic compounds, highlighting physiological traits that may facilitate persistence under the challenging conditions of the nectar environment.

Together, these findings demonstrate how dispersal by insect vectors and physiological adaptation interact to shape yeast communities in a specialized floral habitat.

# Adaptation for Bioremediation: A Mycological Approach

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Heavy metal and radionuclide contamination can still be found at sites previously active in industrial resource extraction and processing. Many of the affected sites require remediation to restore the ecological system to a stable and healthy balance. One promising candidate for this remediation process is the use of basidiomycete fungi, which previous studies have shown can survive and tolerate heavy metal stress while also reclaiming, stabilizing, and diluting otherwise toxic compounds in the affected areas.

The area of particular interest for this application is the former uranium leaching heap near Ronneburg, Thuringia, Germany. Previous studies by Traxler et al. (2019) demonstrated how the white-rot basidiomycete *Schizophyllum commune* can endure the intense environmental pressures of radionuclide-containing soil substrates within the Chernobyl Exclusion Zone (CEZ). Further investigations by Östreicher (2023) examined the endurance and resistance of *Tricholoma vaccinum* to heavy metals. Both fungi therefore served as the focal subjects of this study.

Using strains of *S. commune* and *T. vaccinum* adapted to strontium chloride ($SrCl_2$) and cadmium chloride ($CdCl_2$), respectively, along with their wild-type counterparts, the effects, endurance, and bioremediation potential of these basidiomycetes can be better understood through field inoculation onto radionuclide-containing soil test-field substrates. In parallel with the field inoculation, laboratory-controlled growth assays were performed to examine their growth and endurance under heavy metal stress more closely. Additionally, by investigating changes in the fungal transcriptomes, questions relating to metal-dependent ecological parameters can be better addressed.

# Ectomycorrhizosphere Processes in Soils of former Uranium Mining Sites

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Mycelial networks form enormous surface areas providing three-dimensional interaction structures with soil particles, soil pollutants, microorganisms and plant roots. Particularly processes in the ectomycorrhizosphere bear a potential for mycoremediation of contaminated soils. To develop strategies for soil remediation we work with soil from our test fields on a former uranium mining site near Ronneburg, Thuringia, Germany. Distinct morphotypes of mycorrhizal root tips were identified from the test field, with dominating types and a relatively low diversity. We assume, that soil pollution and acid mine drainage selected for species that were capable to adapt to these challenging conditions. An element analysis of fruiting bodies from the test field showed distinct accumulation patterns. Some rhizosphere bacteria supported mycelial growth of ectomycorrhizal fungi. In a microcosm experiment we analyzed mycorrhizosphere interactions with soil particles. Here, autoclaved test field soil was inoculated with the long-range exploration type ectomycorrhizal fungus *Tricholoma vaccinum*, with and without co-inoculation with its host tree *Picea abies*. For comparison, another fungus able to survive in contaminated soil, the white rot model organism *Schizophyllum commune*, was applied in axenic soil cultures. After 3 months, a rain event was simulated to study the effects of the symbiosis on soil aggregation. Visually, for both fungi mycelial colonization of the soil was observed after that time. Microscopically, the inoculated tree roots showed a very high mycorrhization rate, and the mycelium contributing to soil aggregation by holding together soil particles. Nanoparticle tracking analysis revealed that soil erosion was reduced by the symbiosis only, while axenic cultures of neither fungi nor tree showed such effects. Future analyses include a long-term-incubation of the same set-up including soil moisture measurements and quantification of living/dead hyphae in the soil. These first results of an entirely new experimental design indicate that ectomycorrhizal treatments bear potential for the remediation of former mining sites and might mitigate challenges coming with climate change, such as periods of drought alternating with heavy rain events causing enhanced soil erosion.

# Ecological role of the orsellininc acid (ors) gene cluster in *Aspergillus nidulans*

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Microorganisms are important producers of natural products, that can serve for humans as antibiotics, cancer therapeutics, plant protection agents and several other applications. But of course those products are not produced for human benefit, but because of a selection benefit for in their corresponding ecological niche. In the center of this analysis are the products orsellinic acid, lecanoric acid, and F9775A/B produced by *Aspergillus nidulans*. Those metabolites get produced under several biotic and abiotic stress factors, ranging from arginine starvation up to biotic competition with Streptomycetes, where the cluster gets induced by several arginoketides like azalomycin F. Previous analysis for those compounds could not attribute antimicrobial function in a significant extent, with no antibacterial properties for those compounds and only little activity against some fungal species for lecanoric acid. The question remains – what is their ecological purpose? This study aims to show that those compounds are not produced for a negative selection towards their competitors, but serve as a positive selection tool as a carbon source in a nutrient starved environment, the soil, to enrich beneficial microorganisms. We aim to show that the compounds are able to be digested, enrich bacteria and thus serving as a change in the microbial community, which leads to benefits for *A. nidulans* in it's niche.

# Effect of bacterial consortia on the growth of *Tuber borchii*

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Truffles are hypogeous ascomycetous fungi belonging to Tuber genus, which form ectomycorrhizal association with oak, hazel and beech. The edible mushrooms are known for their unique flavor, smell and cultivating them is quite expensive. The role of microbial communities associated with truffles are important for their flavor, but further roles are rather unknown. The aim of the project is to understand the effect of soil and microorganisms on cultivation of Tuber on truffle field sites.

Microbial community analysis revealed a distinct distribution of microbial taxa between truffle fruiting bodies and surrounding soil. Proteobacteria were predominantly associated with the gleba and peridium of the fruiting bodies, whereas soil samples were dominated by Proteobacteria together with *Actinomycetia, Mortierellomycetes* and *Sordariomycetes*. A strain collection with 355 microorganisms was created from soil and different parts of fruiting bodies, which predominantly contained *Streptomyces sp., Pseudomonas sp*., and *Bacillus sp*. Initial interaction experiments demonstrated that species of Proteobacteria and Actinobacteria induced faster growth of truffle aerial mycelium than control. Based on these findings, selected truffle helper bacteria were combined into consortia and applied to truffle field sites. Long-term observations indicated an increase in soil microbial diversity following the application of both commercially available truffle growth enhancers and laboratory-developed bacterial consortia.

Ongoing greenhouse experiments with *Pinus sylvestris, Betula pendula*, and *Picea abies* are evaluating the combined effects of bacterial consortia on host tree development and truffle colonization. In parallel, species-specific primers for Tuber sp. are being developed to quantify truffle abundance in soil samples and assess establishment success under different treatment conditions.