POSTER ABSTRACTS

2022 Westerdijk Spring Symposium

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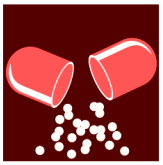

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Collecting information on soil fungal communities in the Andes in the perspective of climate change

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Soil fungi are an essential component of ecosystems, crucial for their role in the nutrients cycles and for their relationships with other organisms. The tropics host a wide biodiversity of soil fungi, but for some areas it is still mostly unknown. The delicate equilibrium of soil microorganisms could be altered by climate change and it is important to study and monitor the situation. Thanks to our international collaborations, we studied the soil mycobiota of two different areas in the South American Andes: the Aguarongo forest reserve in Ecuador and two high altitude pear orchards in Colombia. The metabarcoding of soil samples allowed an in-depth analysis of the whole fungal community of the two areas. In the samples from the Aguarongo forest, 987 OTUs (Operational Taxonomic Units) were identified, belonging to seven different phyla, where the most abundant were *Ascomycota* (32–36%), *Mortierellomycota* (25–28%), and *Basidiomycota* (9–11%). In the samples from the Colombian pear orchards, 629 OTUs were detected, and they were dominated by fungi belonging to *Ascomycota* (64%), *Mortierellomycota* (27%), and *Basidiomycota* (8%). It is important to understand the ecological function, which remains mostly unknown, of the high abundance of the *Mortierellaceae* family in both forest and agroecosystem in the Andes. Many of the species found in both areas were first records of these fungi in Colombia or in Ecuador; a sign of how little is known about soil fungi in these areas and how important it is to carry on research in this field. Moreover, many identified fungal species were bioactive fungi with beneficial activities, such as biocontrol agents, plant-growth promoters and producers of valuable substances. Collecting this information about soil fungal communities is the first step to preserve and monitor them, especially in high altitude regions of the tropics, where the effect of climate change can be extreme, with a shift from psychrophilic to mesophilic species and the introduction of new fungal pathogens due to the change in vegetation.

Genetic diversity of the emerging human fungal pathogen *Pichia norvegensis*

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Pichia norvegensis (= *Candida norvegensis*) is increasingly isolated from hospital settings, especially from immunocompromised patients. Understanding this rare pathogen, including its emergence and distribution, is crucial for accurate diagnosis and infection prevention. We studied the genetic diversity of a large collection of clinical *P. norvegensis* isolates obtained from Dutch hospitals along a set of non-Dutch clinical and environmental isolates.

Clinical (*n*=236; 90.8%) and environmental (*n*=24; 9.2%) *P. norvegensis* isolates were subjected to Amplified Fragment Length Polymorphism (AFLP) fingerprinting and a novel six-loci microsatellite typing panel. Data was analysed with BioNumerics and STRUCTURE. We applied a novel mating-type assay to determine the *MAT*α locus presence/absence.

AFLP fingerprinting separated the *P. norvegensis* isolates into three main clusters. Two clusters fully consist of clinical isolates, the third represented a mix of clinical and environmental isolates. By microsatellite typing the overall genetic diversity was low (Simpson's *D*=0.90), due to the large number of Dutch clinical isolates with similar genotypes. Minimum spanning tree-analysis showed that Dutch clinical isolates fell into two clusters. Environmental and non-Dutch isolates were more distantly related. STRUCTURE analysis showed the presence of four genotypes, with signs of genetic admixture between geographic locations and environmental/clinical isolates. Nearly all isolates harbor the *MAT*α mating-type allele.

The *P. norvegensis* isolates obtained from Dutch hospitals appeared to be largely clonal, independent of geographic origin and isolation date. The observed clonality is supported by the extensive number of *MAT*α isolates. Microsatellite typing indicated potential admixture between clinical and environmental isolates.

New insights in the pathogenic *Trichosporon* species intraspecific diversity: a comparative analyses of IGS1 sequencing and AFLP fingerprinting

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Trichosporon species are ubiquitous basidiomycetous yeast-like, currently recognised as opportunistic pathogen able to cause invasive infections mainly in neutropenic or critically ill patients exposed to invasive medical procedures, and submitted to broad-spectrum antibiotic therapy (Colombo *et al*., 2011; Francisco *et al*., 2019; Nobrega de Almeida *et al*., 2021). The *Trichosporon* taxonomy has been extensively revised, and based on molecular studies 12 species are currently accepted (Liu *et al*., 2015; Takashima *et al*., 2020), namely: *T. asahii, T. asteroides, T. coremiiforme, T. faecale, T. inkin, T. ovoides, T. aquatile, T. caseorum, T. japonicum, T. lactis, T. insectorium* and *T. dohaense.* Molecular typing of *Trichosporon* is currently based on solely the IGS1 ribosomal DNA locus, while a large intraspecific diversity has been observed within *T. asahii* and *T. faecale* (Iturrieta-González *et al*., 2014; Almeida *et al*., 2016; Francisco *et al*., 2021). The validation of these genotypes by robust molecular typing tools is crucial to better characterise their epidemiology.

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Enzymatic activity of faeces-derived *Candida albicans* strains correlates with abdominal pain scores and faecal calprotectin in patients with inflammatory bowel disease in remission

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Treatment of abdominal pain in quiescent inflammatory bowel disease, which occurs for 39% of patients, remains a major unmet clinical need. A role for the luminal gut mycobiome with respect to abdominal pain was previously shown in patients with Irritable Bowel Syndrome (IBS), and causality was confirmed in IBS-like rodents. In addition, *Candida albicans* and its genotypic and phenotypic variations were recently highlighted in relation to abdominal pain. We hypothesise that similar mechanisms may be present for patients with quiescent IBD with abdominal pain. Here, we aimed to correlate phenotypic variation of *Candida albicans* strains with abdominal pain for patients with IBD in remission. Faecal material was obtained from patients with quiescent IBD (defined as faecal calprotectin ≤ 250 µg/g faeces) with (n=91) and without (n=58) abdominal pain determined according to the Irritable Bowel Syndrome Severity Scoring System (IBS-SSS) or Gastrointestinal Symptoms Rating Scale sub-score 'Abdominal pain' (GSRS-AP). *Candida albicans* growth was observed in patients with (n=17) and without (n=12) abdominal pain. Release of proteinase, (phospho)lipase, and esterase was determined by measuring halo diameters on enzyme-specific media substrates for one strain per individual. Correlations between patient data and enzyme activity were made based on Spearman correlations.

While no significant differences in enzyme release were determined between the groups of patients with versus without abdominal pain, correlations between enzyme release and clinical data were observed. Release of protease and phospholipase enzymes (R=0.45, p=0.007) correlated positively. Concerning clinical data, a significant correlation was observed between release of phospholipase and the GSRS-AP sub-score (R=0.83, p=0.02), as well as between protease release and the IBS-SSS (R=0.54, p=0.03). Lastly, a negative correlation was determined for faecal calprotectin levels and lipase enzyme release (R=-0.44, p=0.02). In the current cohort, release of virulence-associated enzymes correlates with clinical scores for abdominal pain and inflammation. Whether these correlations are of relevance in a clinical setting remains to be determined through additional investigations.

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Additions to the genus *Pseudocercospora*

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As one of the largest genera in *Mycosphaerellaceae*, *Pseudocercospora* Spegazzini is a well-known genus with huge species diversity and a destructive impact in agriculture, horticulture and forestry. Being a cosmopolitan genus, *Pseudocercospora* contains more than 1,500 species names, many of which are pathogens, endophytes or saprobes. Important plant pathogens include taxa associated with Sigatoka disease on banana, angular leaf spot of bean, husk spot of macadamia, and Cercospora leaf spot of olive, cactus, avocado and eucalypts. Some species are regulated quarantine pests in the EU, such as *P. angolensis* causing fruit and leaf spot disease on citrus and *P. pini-densiflorae* (as *Mycosphaerella gibsonii*) causing brown needle blight of pine. Phylogenetic analyses of multi-locus DNA sequence data have revealed a high level of host-specificity in *Pseudocercospora*. In the present study, a multi-locus phylogenetic analysis was conducted based on the internal transcribed spacer regions of the nrDNA cistron (ITS), partial actin (*actA*) gene, partial translation elongation factor 1-alpha (*tef1*) gene, and the partial DNA-directed RNA polymerase II second largest subunit (*rpb2*) gene, including 355 newly sequenced strains and 366 reference strains representing more than 300 taxa. Based on these phylogenetic analyses and morphology, more than 35 novel taxa were identified from 9 countries and 30 different hosts.

Expression analysis of transcription factor encoding genes in *Parastagonospora nodorum*

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Transcription factors (TFs) as regulatory elements of gene expression are required for fine-tuning cellular functions during morphogenesis, reproduction, pathogenesis and stress response in fungi. The fungus *Parastagonospora nodorum* is a necrotrophic fungal pathogen causing Stagonospora glume blotch (SGB) disease of wheat. Genome mining of *P. nodorum* revealed that it harbors 486 putative transcription factors (TFs) which represent almost 3% of the total annotated genes. Despite the presence of a high number of TF-encoding genes within the genome of *P. nodorum*, very little is known about their expression profile as well as their function during cellular and biological processes. In this study, we selected 10 putative TF genes: four share homology with well-known fungal TFs involved in pathogenesis; and the other six genes are located within secondary metabolite biosynthetic gene clusters (BGCs). We investigated the expression profile of these candidate TF genes in *P. nodorum* using semiquantitative RT-PCR and quantitative qPCR assays under *in planta* and *in vitro* conditions. We found that six candidate TF genes exhibit the highest expression level at three days post inoculation and in starvation media lacking both nitrogen and carbon sources. Two of these TF genes are found in BGCs that show similarities with BGCs involved in choline and duclauxin production in other fungi, pointing at potential new virulence factors in *P. nodorum*. These findings suggest that these TFs may play an important role in pathogenicity of *P. nodorum*, but their exact function remains to be addressed through functional characterisation with gene knockouts.

Diverse secondary metabolism gene clusters of cereal blast fungus are associated with specialization of host plants

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The ability of a pathogen to break the natural resistance and infect a host depends on its repertoire of effector molecules associated with virulence. Phytopathogens can constantly evolve and deploy a novel effector(s) to adapt to a new host plant. Most plant-pathogen interaction studies thus far have been focused on protein effectors. However, chemical effectors that can shape the plant-microbe interactions have not been well studied so far. Here, we mined 71 genomes, belonging to various lineages of a multi-host plant pathogen *Pyricularia oryzae*, for secondary metabolites producing biosynthetic gene clusters (BGCs). The similarity network analysis showed a significant diversity among a total of 4224 BGCs from these lineages of *P. oryzae*. The BGCs, based on similarities in content and architecture of the gene cluster, were grouped into gene cluster families (GCFs). Interestingly, we found that a few GCFs were unique to different host-specific lineages of *P. oryzae*. Further, elucidation of phylogenetic relationships within core biosynthetic genes identified a novel reducing polyketide synthase (PKS) gene distinctly present in the lineage adapted to rice host. We hypothesize that the novel evolutionarily acquired PKS produces a secondary metabolite that likely acts as a chemical effector associated with adaptation *of P. oryzae* to rice host. Our research findings highlight the need to explore and characterise more such chemical effectors involved in host-pathogen interactions, which in turn will shed light on the metabolic strategies used by a pathogen to adapt to a new host.

Revisiting *Clonostachys*

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Clonostachys (*Bionectriaceae, Hypocreales*) species are common soilborne fungi that also occur as mycoparasites and in plants as endophytes, epiphytes, and saprotrophs. The sexual morphs of *Clonostachys* are members of *Bionectria*, which was subdivided into six subgenera, *Astromata*, *Bionectria*, *Epiphloea*, *Myronectria*, *Uniparietina* and *Zebrinella*. However, with the introduction of the One Fungus-One Name concept the genus *Clonostachys* was recommended for conservation over *Bionectria*. Species of *Clonostachys* are typically characterised by penicillate, frequently sporodochial, and, in many cases, dimorphic conidiophores (primary and secondary conidiophores). Primary conidiophores are mononematous, either verticillium-like or narrowly penicillate, and form heads of slimy conidial masses. Secondary conidiophores can be mononematous, loosely aggregated, and generally form imbricate conidia that can collapse to slimy masses that vary from white, to pale orange, yellowish or green. In the present study we investigate the species diversity within a collection of 458 strains of *Clonostachys* deposited in the CBS culture collection based on morphological and DNA sequence analyses of the nuclear ribosomal internal transcribed spacer (ITS), partial sequences for the 28S large subunit (LSU) nrDNA, partial RNA polymerase II second largest subunit (*rpb2*) and partial translation elongation factor 1-alpha (*tef-1α*) gene regions. Based on these results, the subgenera *Astromata*, *Bionectria*, *Myronectria* and *Zebrinella* are supported within *Clonostachys*. The genus *Sesquicillium* is resurrected for the subgenera *Epiphloea* and *Uniparietina*. One new genus, 30 new species and several new combinations are proposed based on results of this study.

UNITE: species hypothesis matching analysis and organisation updates

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The new UNITE species hypotheses (SH) matching analysis is a digital service for species discovery from environmental DNA. The service is based on UNITE datasets (https://unite.ut.ee) hosted in PlutoF (https://plutof.ut.ee). Its output includes information on what SHs are present in eDNA samples, whether they represent potentially undescribed species, other studies that have found the same SHs, and more. The output provides DOI (digital object identifier)-based stable identifiers for unambiguous communication of species found in eDNA. DOIs are connected to the taxonomic backbone of PlutoF and GBIF (https://www.gbif.org). In this way, every DOI is accompanied by a taxon name that is used simultaneously for the communication of species. In the case of potentially undescribed species or species that are not present in the UNITE SH system, preliminary DOIs are issued by the PlutoF system. UNITE services are focused on fungi but cover all *Eukarya* by using publicly available rDNA ITS marker sequences and accompanying sample metadata. Analysis results can be published in GBIF as DNA-derived occurrence data. The source code of the UNITE SH matching analyses is freely available in GitHub (https://github.com/TU-NHM/sh_matching_pub). Further information about the UNITE resources is provided in the references below.

The UNITE Community became a non-profit association in January 2022. It is governed by a general meeting of the members of the association, which in turn elects a management board. UNITE's advisory board will be formed in the fall of 2022. UNITE Community will also invite new members to join the association.

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dnabarcoder: an open-source software package for analyzing and predicting DNA sequence similarity cut-offs for fungal sequence identification

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The accuracy and precision of fungal molecular identification and classification are challenging, particularly in environmental metabarcoding approaches as these often trade accuracy for efficiency given the large data volumes at hand. In most ecological studies, only a single similarity cut-off value is used for sequence identification. This is not sufficient since the most commonly used DNA markers are known to vary widely in terms of inter- and intra-specific variability. We address this problem by presenting a new tool, dnabarcoder, to predict similarity cut-offs and resolving powers of a biomarker in different clades of fungi for sequence identification. It was shown that the predicted similarity cut-offs varied significantly between the clades of a recently released ITS DNA barcode dataset from the CBS culture collection of the Westerdijk Fungal Biodiversity Institute. When classifying a large public fungal ITS dataset – the UNITE database – against the barcode dataset, the local similarity cut-offs assigned fewer sequences than the traditional cut-offs used in metabarcoding studies. However, the obtained accuracy and precision were significantly improved. Our study also showed that it might be better to extract the ITS region from the ITS barcodes to optimise taxonomic assignment accuracy. Furthermore, 15.3%, 25.6%, and 26.3% of the fungal species of the barcode dataset were indistinguishable by full-length ITS, ITS1, and ITS2, respectively. Except for these indistinguishable species, the resolving powers of full-length ITS, ITS1, and ITS2 sequences were similar at the species level. Nevertheless, the complete ITS region had a better resolving power at higher taxonomic levels.

Natural products of the mushroom *Gymnopilus imperialis* - A metabolomic and genomic approach

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Gymnopilus consists of a widely distributed genus of mushroom-forming fungi, especially in tropical regions of the world. Literature on *Gymnopilus* representatives reports the presence of oligoisoprenoids, and styrylpyrones (Hatfield & Brady, 1969). Considering the large number of secondary metabolites that basidiomycetes might contain, dereplication tools such as GNPS (Global Natural Products Social Molecular Networking), have become important in prospecting metabolites, saving time and work on isolation and characterisation of natural products. Thus, this work identified the wild mushroom *Gymnopilus imperialis* and dereplicated its extracts with the aid of GNPS to annotate oligoisoprenoids. It was possible to annotate 24 oligoisoprenoids from methanol, dichloromethane, and ethyl acetate extracts of *G. imperialis,* four of them from GNPS spectral library match, and 20 from the prediction based on a molecular network. Moreover, HRMS-ESI-(+) dereplication of the acetate extract annotated bisnoryangonin and hispidin, hybrid NRPS-PKS molecules. Based on the annotated molecules, the main purpose of this work is to use genomics, metabolomics, and molecular biology to identify PKSs and terpene gene clusters related to the biosynthesis of styrylpyrones and oligoisoprenoids in the basidiomycete *G. imperialis,* according to the evaluation of its genome through a "retro-biosynthetic" approach. Genome and transcriptome analyses obtained from this species will reveal which genes have been expressed and which are silent (Mosunova *et al.*, 2020). As the genome mining of *G. imperialis* is still in progress, the biosynthetic gene clusters were predicted in three correlated species *Galerina marginata, Gymnopilus chrysopillus* and *Gymopilus junonius* using fungiSMASH (Blin *et al*., 2019). These predictions were further analysed using the BGCtoolkit software (unpublished) that is being developed in the Fungal Natural Products group. Phylogenetic and comparative genomics analyses were performed to identify candidate pathways for already reported or novel pyrones. According to these analyses, it was possible to select 10 biosynthetic clusters candidates for heterologous expression, seven of them being sesquiterpene synthases, and three PKSs. Future perspectives of this project involve the heterologous expression in *Aspergillus oryzae* of the clusters and screening for the production of secondary metabolites (Zhang *et al*., 2019).

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Fungal quinone batteries

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Filamentous fungi usually produce a large number of secondary metabolites, which can be candidates for new antibiotics, anti-cancer compounds, antioxidants, pigments, *etc*. Some of these pigments can be used for colouring foods and textiles, but the fungi can also be applied in a sustainable production of quinones, which can be used in batteries. The most effective producers secrete such quinones in large amounts for sustainable rinse up. The *Penicillium* species *P. phoeniceum*, *P. atrosanguineum*, *P. manginii* and *P. chermesinum* all secrete the red bibenzoquinone phoenicine of use in fungal quinone batteries. By adding extra sucrose to the growth media tested, *P. phoeniceum* secreted very large amounts of phoenicine (approximately 5 g/l) without any other optimisation of the natural producer. Many other *Penicillium*, *Aspergillus*, *Talaromyces* and other filamentous fungi produce benzoquinones, naphthoquinones and / or anthraquinones, where efficient and sustainable quinone production and purification requires secretion of the secondary metabolite (Christiansen *et al*., 2021). Fungal quinone batteries can be used for sustainable long time storing of energy from the weather-dependent windmills and solar panels in the future.

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Elucidation of pyranone pigment biosynthesis in fungi

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Industrial production of dyes relies on petrol-based methods with corrosive chemicals that are harmful to both environment and humans. While the dyestuff industry and its derivatives (textile, paint, *etc*.) greatly contributes to the economic growth on the short term, they cause serious pollution issues that are detrimental over the long term. Hence, we urgently need new sustainable production routes. Dyes of natural origin represent an attractive alternative. Especially, pigments produced by fungi are promising because these microbes can be used as cell factories to produce natural dyes in fermentation-based processes. The chemical family of pyranone compounds produced by fungi exhibit diverse colours and biological activities. Xylindein is a natural cyan pigment produced by the fungus *Chlorociboria aeruginascens*. In this study, we re-sequenced *C. aeruginascens* and mined the genomes to identify candidate gene clusters for the production of xylindein. We assessed expression of the candidate genes and have embarked on the functional characterization using heterologous expression in the industrial host *Aspergillus oryzae*. Elucidation of the pathway will open the possibility to engineer novel pyranone pigments.

Exploring the diversity of Kenyan fungi for the beneficial metabolites and potential biocontrol agents

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The MYCOBIOMICS project, funded by the Horizon 2020 Marie Skłodowska-Curie Research and Innovation Staff Exchange (RISE), aims to use mycobiota from Asia, Africa and Europe to search for beneficial metabolites and potential biocontrol agents using OMICS techniques. With partners specialising in applied mycology, the project has researched beneficial secondary metabolites for antibiotic development and identified potential candidates for the development of eco-friendly fungal biocontrol agents. The project will also characterise the mycobiota of important agricultural plants. Fieldwork involves collecting fungi from soil, animal dung, plants and insects in Kenya, South Africa and Thailand. Isolated fungal strains are selected based on taxonomy and biological activity screening. Strains belonging to new or poorly explored lineages that show biological activity are subjected to further study with the aim of discovering new bioactive molecules and studying the background of their production. The project aims to exchange knowledge between students and researchers through secondments and mutual training. The poster presents the isolation, identification and biologically activity screening of fungi isolated from soil and bark beetles in different biotopes of Kenya.

Novel fungi described at the BiMM research platform (Tulln, Austria) since 2018

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The research platform Bioactive Microbial Metabolites (BiMM) was established in 2015 as a high-throughput approach to discover novel bioactive compounds by applying interactions between bacteria and filamentous fungi (co-cultivations). The microbial strains used in co-cultivations have been isolated by our team mostly from an environment (soil, water, air) or from clinical (veterinary and human) settings. During these microbial surveys, several new fungal taxa have been revealed and described based on the polyphasic approach. *Metapochonia lutea* (*Ascomycota*, *Hypocreales*) isolated from littoral water of the Danube river in Tulln (Austria) in 2017, was the first fungal taxon described by the BiMM team. *Saksenaea dorisiae* (*Mucoromycota*, *Mucorales*) was isolated from a water sample from a private well in Manastirica-Petrovac in the Republic of Serbia sampled in 2018. Four new *Keratinophyton* species (*Ascomycota*, *Onygenales*) were described in 2021, namely *K. gollerae* and *K. wagneri* isolated from forest soil (Tatranská Lomnica, SK, in 2019 and 2015, respectively), *K. lemmensii* from compost soil (Tulln, AT, in 2015), and *K. straussii* from garden soil (Vieste, I, in 2015). In the same year, two new toxigenic members of *Penicillium* section *Exilicaulis* (*Ascomycota*, *Eurotiales*) were described, namely *P. krskae* isolated from air as a lab contaminant in Tulln (AT) and *P. silybi* isolated as an endophyte from asymptomatic milk thistle stems in Josephine County (Oregon, USA). In addition to these already published taxa, two novel ascomycete species belonging to *Gymnascella* (*Onygenales*) isolated from a dust sample from a cow stable in Austria in 2021 and *Penicillium* (*Eurotiales*) from a freshwater stream in Connecticut (USA) in 2019 have been submitted to taxonomical journals (Sydowia and Fungal Planet, respectively).

Characterisation of cytochalasins isolated from *Xylariaceae* and description of their bioactivity on the eukaryotic actin cytoskeleton

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Members of the *Xylariaceae* (*Ascomycota*, *Xylariales*) are well known producers of the cytochalasans, hybrid secondary metabolites synthesised by cooperation of a non-ribosomal peptide synthetase and a polyketide synthase with over 200 described naturally occurring derivatives described to date. Cytochalasans are commonly used as tool compounds to examine cell biological processes, as they are widely used for their ability to affect the eukaryotic actin cytoskeleton by blocking its dynamic polymerisation. As the stability and function of the actin network and its dynamics depend on its constant turnover, many essential motile processes such as migration, cytokinesis or the stability of the plasma membrane are affected. This has been previously rationalised to be caused by the capability of cytochalasans to bind at the barbed end, the fast-growing end, of filamentous actin (F-actin), which ultimately leads to its extensive depolymerisation throughout a living cell. While macroscopic effects are well documented, details about the influence of structural deviances of cytochalasins and the exact chain of events leading to the collapse of the F-actin network remain poorly understood. Here, we report the results of a taxonomically informed antimicrobial screening campaign focusing on members of the *Xylariaceae* and the isolation of different cytochalasins produced in solid and liquid culture. Secondary metabolite production was tracked by liquid chromatography coupled to a diode array electrospray mass spectrometer (HPLC/DAD-ESIMS), adequate media selected and fermentation scaled up to yield sufficient amounts for liquid chromatography in preparative scale to afford pure compounds for their structural assignment by nuclear magnetic resonance spectroscopy (NMR). Bioactivity on tissue culture cells was assessed using a metabolic activity test (MTT) testing for toxic concentrations of the isolated compounds, and a recently developed F-actin disruption assay to examine possible differences in the activity spectrum. The F-actin network of treated and fixed cells was stained with fluorescently coupled phalloidin to enable the evaluation of the extent of F-actin network disruption. We here demonstrate a systematic, comparative approach to isolate and categorise active natural compounds from different sources with the aim to exploit their complete range of bioactivities.

Glycoside Hydrolase family 30 harbors fungal subfamilies with distinct polysaccharide specificities

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Glycoside hydrolases are critical for the enzymatic hydrolysis of plant biomass, and belong to different families in the Carbohydrate-Active enZyme (CAZy) database (Lombard *et al*., 2014). Glycoside Hydrolase family 30 (GH30) is a diverse but understudied family, containing enzymes from bacteria, fungi and animals. To deepen our understanding of the fungal members in GH30, we used more than 150 amino acid sequences to construct a GH30 phylogenetic tree, of which eleven candidates covering different fungal subfamilies (SFs) were selected for biochemical characterisation using polysaccharides and crude plant biomass.

Our phylogenetic analysis of GH30 updated the fungal SFs (GH30_SF3, SF5, and SF7) included in the CAZy database. Fungal members were clustered in SF3, SF10, SF5, and SF7. Functional characterisation revealed that enzymes from different SFs exhibited distinct substrate specificities. However, while the different subfamilies act on different polysaccharides, they all mainly release 'short' non-digestible di- and oligosaccharides, which could be of interest in food and feed industries. One new xylobiohydrolase shows high potential for commercial xylobiose production. Additionally, a new fungal subfamily in GH30 was proposed, *i.e.* GH30_11, which displays β-(1→6)-galactobiohydrolase. These findings contribute to understanding the fungal GH30 subfamily and facilitate industrial applications of fungal GH30 enzymes.

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Development of novel fungal cell factories for applications of biobased economy

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Filamentous fungi have been utilised to produce a wide range of valuable compounds for centuries. With the advent of molecular biology and biotechnology, these fungi have been exploited as expression hosts for the production of primary and secondary metabolites, enzymes, and homologous and heterologous proteins which are worth several billion dollars per year. However, compared to homologous proteins, the production levels of heterologous proteins are far from optimal. *Aspergillus vadensis* is unique in that it does not acidify the growth medium and produces very low levels of extracellular protease activity, and is therefore ideally suited for the production of heterologous proteins. We will develop this species into an efficient and versatile cell factory.

Genome wide insights into signal integration by the G-protein pathway for regulation of carbon- and secondary metabolism in *Trichoderma reesei*

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Nutrient sensing is of utmost importance for gene regulation in fungi, with the heterotrimeric G-protein pathway as prototypical transmission machinery. Previously we could show an interrelationship between light response and cellulase gene regulation in the filamentous fungus *Trichoderma reesei*. Recently we found that also secondary metabolism is regulated in a light dependent manner in *T. reesei*. In both cases, G-protein coupled receptors (GPCRs) exemplified these connections: the glucose sensors CSG1 and CSG2, which are responsible for posttranscriptional regulation of cellulase expression as well as GPR8, a GPCR associated with the sorbicillin cluster.

To gain further insight into the balance between carbon- and secondary metabolism along with its dependence on light, we performed functional, transcriptome analyses and network analysis. We used deletion mutants Δ*gna1*, Δ*gna2* and Δ*gna3,* Δ*gnb1* and Δ*gng1* as well as strains expressing constitutively activated versions of the G-alpha proteins, GNA1QL, GNA2QL and GNA3QL in *T. reesei* QM6a. We found characteristic alterations in biomass formation, enzyme production and growth as well as an influence on sexual and asexual development. Cellulase gene transcription was differentially regulated between the investigated G-protein mutant strains with important differences between light and darkness. Analysis of secondary metabolite production revealed an impact on regulation of several compounds hence substantiating the link between cellulase regulation and secondary metabolism.

We conclude that the G-protein pathway integrates signals relevant for carbon- and secondary metabolism to optimally balance enzyme biosynthesis and growth with metabolite production.

First insight into *Chaetosphaeriaceae* biology using genomic data

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The species of the *Chaetosphaeriaceae* (*Chaetosphaeriales*, *Sordariomycetes*, *Ascomycota*) are microscopic asexually reproducing fungi, some of which belong to the life cycle of sexual morphs with macroscopic fruit bodies (~300 µm diam) perceptible as "black dots" on the surface of decaying plants. They are invisible to most of the people and difficult to find even by a trained eye. In addition, they are usually hard to cultivate and slow growing, which make them difficult to study. Traditionally, they are reported from the decaying plant matter in the forest habitat and some exhibit an endophytic lifestyle (Réblová *et al*., 1999). Surprisingly, analysis of metabarcoding data showed that they are also common soil fungi, dwelling in a bulk soil in grasslands and croplands. There are about 44 genera classified into this family (Wijayawardene *et al*., 2020) with monographs available for several genera, but with very little knowledge about their biology. Studies showed that *Chaetosphaeriaceae* members are important degraders of lignocellulose in wood, litter and soil and have rich structural diversity of natural compounds (*e.g.* terpenoids, cyclic peptides, naphthaquinones, lactones, lipopeptides, xanthones). Three unpublished and unannotated draft genomes of *Chaetosphaeriaceae* are available (1,000 fungal genome project). Nevertheless, the information about the genetic background of the basic pathways is missing in this fungal lineage. The same is true for the whole *Chaetosphaeriales*. Our study is filling this gap using analysis of genomes from 16 species.

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Urban soil fungal biodiversity: the citizen science projects at the Westerdijk Institute

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Preserving biodiversity is a hot item these days and the Westerdijk Institute (WI) has launched a very successful citizen science project in order to increase awareness of biodiversity among school children and to promote the collection of new fungi from Dutch soils. Visitors of the University Museum of Utrecht University obtained a sampling kit, and could subsequently submit a soil sample from their home garden to the institute. Upon analysis, researchers from the WI identified which fungi were present. New fungi were named after the person or family or school who collected it. Therefore, the project was termed '*World fame, a fungus with your name'*. Because of the overwhelming success, we continued with a second project, '*Fungi for the future*', in which further soil samples were analysed. This time we contacted science teachers from specific schools in The Netherlands, and one from Belgium. From the 400 soil samples that were submitted to the WI, we isolated 4000 fungal strains. High biodiversity has been found with more than 600 species recorded distributed in several classes: *Sordariomycetes*, *Dothideomycetes*, *Eurotiomycetes*, *Leotiomycetes*, *Mortierellomycetes*, *Umbelopsidomycetes*, *Tremellomycetes*, *Saccharomycetes*, *Mucoromycetes*, *Agaricomycetes*, *Orbiliomycetes*, and *Basidiomycetes*. Currently, one new fungal family, five genera, and 51 species have been identified during these citizen science projects (Crous *et al.* 2017, 2018, 2021; Groenewald *et al.* 2018; Giraldo *et al.* 2019, Hernández-Restrepo *et al.* 2020, Hou *et al.* 2020). This number will still increase significantly, since there are many other fungal species that could as yet not be identified, and are currently being studied by different researchers.

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Diversity of *Akanthomyces* (*Cordycipitaceae*) on adult moths in Thailand

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During a survey of entomopathogenic fungi on adult moths (*Lepidoptera*) in Thailand, investigations using molecular phylogeny based on multi-gene loci comprising the partial sequences of the ribosomal DNA large subunit (LSU), translation elongation factor-1-α (*TEF*), the largest and second largest subunits of the RNA Polymerase (*RPB1*, *RPB2*), as well as studies of important morphological characters revealed six new species of *Akanthomyces* on a wide variety of moth families. These new species include *Akanthomyces buriramensis*, *A. filiformis*, *A. fusiformis*, *A. niveus*, *A. obovoideus* and *A. phariformis*. In a previous study of *Akanthomyces* species on moths in Thailand, three species were described occurring on specific moth families, such as *A. noctuidarum* found only on the moth family *Noctuidae*, *A. pyralidarum* on *Pyralidae* and *A. tortricidarum* on *Tortricidae* moths. Some new species in this study showed broader host ranges, *e.g.*, *A. buriramensis* was found occurring on both *Noctuidae* and *Notodontidae*, *A. filiformis* on unidentified *Lepidoptera* and *Pyralidae*, *A. phariformis* on *Erebidae* and *Noctuidae*, and *A. obovoideus* on *Arctiidae*, *Erebidae* and *Lymantriidae*. Only *A. fusiformis* and *A. niveus* are found only on *Pyralidae*, and both species differ morphologically with *A. pyralidarum* that was first reported occurring on this family. The new and known species are illustrated.

Ancestral traits in the genomes of *Mucoromycota* fungi

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Mucoromycota is a diverse group of fully terrestrial fungi often associated with plant-material. Their ecological significance is hard to be overestimated yet this lineage is relatively understudied. In our studies, we try to fill this knowledge gap by screening publicly available and newly sequenced genomes. We found that *Mucoromycota* differs from *Dikarya* in many genomic features. They harbour transposons of families rarely found in *Dikarya* including Merlin, Sola and P DNA transposons (Muszewska *et al*., 2017), and DIRS retrotransposons (Muszewska *et al*., 2013). We demonstrated that they encode a profile of secondary metabolite clusters with the dominance of terpenoid and NRPS-like clusters (Koczyk *et al*., 2021). Unexpectedly, we revealed that they possess genes involved in cobalamin metabolism which were apparently lost in plants and *Dikarya* (Orłowska *et al*., 2021). Their lipid composition includes gamma-linolenic acid, which can be a chemotaxonomic marker. *Mucoromycotina* is of particular interest for lipidome studies because of the high share of unsaturated fatty acids which have biotechnological and nutritional value. These fungi have a high share of fucose in their chitin-chitosan cell walls (Muszewska *et al*., 2021) which is not present in the younger fungal lineages. Moreover, they encode an array of fucosyltransferases working on diverse substrates. The fucose metabolic network may resemble in its complexity the one present in animals.

Most of these traits are likely inherited from ancestors, shared with *Metazoa* and contribute to the biology of *Mucoromycota*. Our results extend the list of traits separating model *Ascomycota* from early-diverging fungal lineages.

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Five new insect pathogenic fungi occurring on scale insects in Southeast Asia

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Five new species of fungi associated with scale insects found in national parks and community forests in Thailand are described based on morphological characteristics and phylogenetic analyses. These new species are characterised by the host, sizes, colors of stromata, and multi-septate conidia production. Morphologically it resembles *Ascopolyporus* and *Hyperdermium,* as well as *Cordyceps piperis* in *Cordycipitaceae*. Phylogenetic analyses of the partial regions of the nuclear ribosomal large subunit (LSU), the translation elongation factor 1-α (*TEF1*), and the largest and second largest subunits of RNA polymerase II (*RPB1* and *RPB2*) recognised these five new species that are nested within the clade of *Ascopolyporus* spp. including the type species of *Hyperdermium, H. caulium*. Our results suggest that the specimens in this study clearly belong to *Ascopolyporus*, consisting of *Ascopolyporus albus, A. galloides, A. griseoperitheciatus*, *A. khaoyaiensis* and *A. purpuratus*. Additionally, the position of *H. pulvinatum* and *C. piperis,* which are clustered together and clearly separated from *H. caulium*, permits the transfer of these two species to a new genus *Neohyperdermium*.

Discovering the Fungal Tree of Life: Methods for uncovering fungarium diversity

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Constructing the fungal tree of life is a colossal undertaking which will require collaboration from a global mycological community. Kew's Discovering the Fungal Tree of Life project is an ambitious program aiming to utilise fungarium collections to add whole genome sequence data for underrepresented fungal groups to a backbone fungal tree of life. With over 1000 fungarium specimens targeted, sampled, and extracted, the project also provides insight into methodological pitfalls and advantages associated with large scale tree of life projects, and their use of natural history collections. Whilst the whole genome data produced by this project will be used for traditional phylogenetic purposes, analysis of by-catch sampling and DNA quantity and quality data allows us to uncover patterns and propose best practice for future projects. Here we present the preliminary results from analysis across the Discovering the Fungal Tree of Life pipeline that highlights methods for sequencing whole genomes from a broad range of taxonomic groups, including some case studies from previously underrepresented groups and specimens ranging from freshly collected to over 100 years old.

Comparison of sugar catabolic pathways in six evolutionarily diverse fungi

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Fungi play a critical role in the global carbon cycle by degrading plant polysaccharides to small sugars and metabolising them as carbon and energy source. Obtaining a detailed insight into sugar catabolism of different species is highly relevant for our understanding of the role of fungi in their natural environment as well as many industrial applications. We mapped the well-established sugar metabolic network of *Aspergillus niger* to five taxonomically distant species (*Aspergillus nidulans*, *Penicillium subrubescens*, *Trichoderma reesei*, *Phanerochaete chrysosporium*, and *Dichomitus squalens*) using an orthology-based approach. In addition, we filtered predicted sugar metabolic genes and compared their expression profiles based on extensive transcriptomic data of fungi grown on diverse monosaccharides.

Our results revealed that the diversity of sugar metabolism correlates well with the taxonomic distance of the fungi. The sugar pathways are highly conserved between the three studied *Eurotiomycetes*, except for duplication of individual enzymes in specific species. A larger level of diversity was observed between the *Sordariomycetes* species and *A. niger*, and even more so for the *Basidiomycota* species. These results were confirmed by the transcriptomes and growth profiles during growth of the fungi on the corresponding sugars.

In conclusion, the establishment of sugar pathway models in different fungi revealed the diversity of fungal sugar conversion and provided a valuable resource for the community, which will also facilitate rational metabolic engineering of these fungi as microbial cell factories.

A specific L-arabitol transporter in *Aspergillus niger*

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L-arabitol is an intermediate of the pentose catabolic pathway in fungi but can also be used as a carbon source by many fungi, suggesting the presence of transporters for this polyol. In this study, an L-arabitol transporter, LatA, was identified in *Aspergillus niger*. Growth and expression profiles indicated that LatA is specific for L-arabitol and is regulated by the arabinanolytic transcriptional activator AraR. Moreover, L-arabitol production from wheat bran and sugar beet pulp was increased in a metabolically engineered *A. niger* mutant by the deletion of *latA*, indicating its potential for improving L-arabitol producing cell factories. Phylogenetic analysis showed that homologs of LatA are widely conserved in fungi.

Unravelling the diversity of sugar related reductases in *Aspergillus niger*

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Metabolic engineering of filamentous fungi gained more attention in recent years. Especially in the context of creating better fungal industrial cell factories to produce a wide range of enzymes and valuable metabolites from plant biomass. Recent studies into the pentose catabolic pathway (PCP) in *Aspergillus niger* revealed functional redundancy in most of the pathway steps. Interestingly, this redundancy appears to be even larger when the fungus is grown on plant biomass compared to pure pentose sugars. In this study, we are exploring this redundancy by identifying the function of paralogous genes of the known pathway genes, to reveal the metabolic diversity of *A. niger* and related fungi. Phylogenetic analysis of the PFAM family that contains the known pentose reductases resulted in the identification of five additional genes in *A. niger* with high similarity to the three characterised genes. In this poster the current results will be summarised.

Machine learning prediction of novel pectinolytic enzymes in *Aspergillus niger* through integrating heterogeneous (post-)genomics data

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Pectinolytic enzymes are a variety of enzymes involved in breaking down pectin, a complex and abundant plant cell wall polysaccharide. In nature, pectinolytic enzymes play an essential role in facilitating bacteria and fungi to depolymerise and utilise pectin. In addition, pectinases have been widely applied in various industries, such as food, wine, textile, paper and pulp industry. Due to their important biological function and increasing industrial potential, discovery of novel pectinolytic enzymes has received global interest. However, traditional enzyme characterisation relies heavily on biochemical experiments, which are time consuming, laborious and expensive. To accelerate identification of novel pectinolytic enzymes, an automatic approach is needed.

We developed a machine learning (ML) approach for predicting pectinases in the industrial workhorse fungus, *Aspergillus niger*. The prediction integrated a diverse range of features, including evolutionary profile, gene expression, transcriptional regulation and biochemical characteristics. Results on both the training and independent testing dataset showed that our method achieved over 90% accuracy, and recalled over 60% of known pectinolytic genes. Application of the ML model on the *A. niger* genome led to the identification of 95 pectinases candidates, covering both previously described pectinases and novel pectinases that do not belong to any known pectinolytic enzyme family. Our study demonstrated the tremendous potential of the ML method in discovery of new industrial enzymes through integrating heterogeneous (post-)genomics data.

Characterization of L-xylulose reductase LxrB from *Aspergillus niger*

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Plant biomass is the most abundant carbon source in nature. The structure of the plant cell wall is heterogenous, containing cellulose, hemicellulose and pectin. Hemicellulose is the main source of pentoses – L-arabinose and D-xylose. In most fungi these two pentoses are converted through the pentose catabolic pathway (PCP). This pathway involves a number of reduction and oxidation reactions with final phosphorylation that leads to D-xylulose-5-phosphate. In *Aspergillus niger* this relatively simple cascade of reactions was verified revealing high complexity of the PCP (Chroumpi *et al.* 2021).

In this study we biochemically characterised the second L-xylulose reductase (LxrB) from *A. niger*. This enzyme catalyses the conversion of L-xylulose into xylitol in the PCP. LxrB was heterologously produced in an *E. coli* expression system, purified and biochemically analysed. LxrB is active on a wide range of sugars and polyols, but the highest activity was observed on L-xylulose, L-sorbose and D-galacturonic acid.

Analysis of its expression pattern revealed that *lxrB* is highly expressed on most of the tested carbon sources, but highest on D-xylose. It is under control of the AraR transcription factor.

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Genome-wide prediction and expressional analysis of sugar transporters in four ascomycete species

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Aspergillus niger, *Aspergillus nidulans*, *Penicillium subrubescens* and *Trichoderma reesei* are industrially important filamentous species. Sugar transporters (STs) play a significant role in sugar uptake after the degradation of cellulosic material. However, the genome-wide distribution and expression dynamics of sugar transporter in different fungal species remains poorly understood. In this study, we predicted 342 STs in the four studied ascomycetes through computational search of a conserved protein domain and manual inspection of the predicted sequence in the Transporter Classification Database. Putative STs were further classified into nine subfamilies and their possible sugar substrate was assigned according to phylogenetic analysis. Comparative transcriptomic analysis of STs on different sugars revealed highly complex expression profiles. The STs from same sub-family in the same or different fungi sometimes displayed very different expression patterns. Our study provides new insights into the diversity of STs in different fungi, which will facilitate future biochemical characterization, metabolic engineering and industrial applications of these candidate STs.

Unbiased mass spectrometry reveals the histone code in the fungal genus *Aspergillus*

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The multi-fold nucleoprotein structure, chromatin, controls the access to genetic information in eukaryotes via modifications like methylation and acetylation on histone proteins H2A, H2B, H3, and H4 (Labrador & Corces, 2002). The combination of different histone modifications on one genomic region – the histone code – impacts the chromatin dynamics between the compact and transcriptionally silent state and the loose and transcriptionally active state (Grunstein *et al*., 1995). This dynamic is crucial for organisms to adapt gene expression patterns in response to a variety of developmental and environmental clues. The fungal genus *Aspergillus* comprises at least 350 species, including human or crop pathogens, food contaminants, as well as important cell factories for industrial and medical applications (Samson *et al*., 2014). The histone code has been shown to be the global regulator of gene expression in several Aspergilli, influencing growth, sexual development, secondary metabolite biosynthesis, and virulence (Cánovas *et al*., 2014). According to our previous computational analysis, the evolutionary history of 16 chromatin modifiers (including 86 subunits) has revealed the conservation of most of the catalytic subunits and flexibility of accessory subunits in the fungal genus of *Aspergillus* (Zhang *et al*., 2022). However, we so far lack an in-depth overview of histone modifications in Aspergilli verified by experiments as well as an estimate of the diversity of abundance between different species. Here, we employed unbiased mass spectrometry to determine the pattern of histone modifications in three Aspergillus species (*A. niger*, *A. nidulans* (two strains), and *A. fumigatus*). We detected 25 histone modifications, including mono-, di-, or tri-methylation and acetylation on both the tail and core domain of histone protein, in which19 histone modifications occurred on histone protein H3, with the remaining six on H4. Quantitively, the four strains share a similar relative abundance for most histone modifications, while the three species differ in H3K9me3, H3K36me1, and H3K79me2, the two *A. nidulans* strains differ in H3K4me2, H3K9ac, H3K36me1, and H4 di_ac. In summary, this analysis will pave the way for future research into the complexity of the histone code and its functional implications on genome architecture and gene regulation in fungi.

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The C_2H_2 transcription factor SltA is involved in conidial germination and hyphal elongation in *Aspergillus fumigatus*

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Aspergillus fumigatus is a filamentous saprophytic fungus that produces multinucleate tubular cells termed hyphae. Hyphal tip extension occurs through the synthesis and addition of new cell wall and membrane at the apical plasma membrane. This highly polar extension of the tip helps *A. fumigatus* to penetrate and invade blood vessels and tissue which results in invasive aspergillosis (IA). Before the fungus grows in a highly polarised manner, the conidium breaks dormancy and the reactivated cell expands isotropically before it undergoes localised expansion of the cell membrane which leads to a tubular outgrowth. Potential regulators of germination and early growth remain largely unexplored. We selected fourteen transcription factors (TFs) upregulated during isotropic and/or polarised growth using transcriptomic data from our previous study. TF knock out mutants were generated in the parental strain MFIG001 (WT), which is deficient in non-homologous end joining. We utilised bright-field and fluorescence microscopy to examine conidial germination of the TF null mutants and WT temporally (0 to 12h). We observed a markedly distorted hyphal elongation morphology in the *ΔsltA* mutant that is not apparent in WT strain and other TF null mutants used in this study. The *ΔsltA* mutant had a germination rate almost two times higher after 6 h compared with the WT and Δ*sltA*rec strain. Germination rate was similar after 8 h of growth and reached around 95% in all strains. Hyphae of the *ΔsltA* mutant hyperbranched and some of the branched hyphae annihilated tubular elongation in RPMI-1640 medium. After 72 h the *ΔsltA* strain showed reduced colony growth on Aspergillus Minimal Medium when compared with the WT and *ΔsltA*rec strains. However, when exposed to cell wall stress agents (calco fluor white, congo red and caspofungin) the relative colony size increased in the Δ*sltA* strain compared with WT and Δ*sltA*rec strain. This suggests a role for *sltA* in cell wall biosynthesis and membrane stability. Altogether, we identified a role for the transcription factor *sltA* in germination and tubular growth of the hyphal tip. Additional experiments will be performed to analyse the molecular mechanisms underlying the distorted hyphal elongation phenotype in the *ΔsltA* strain.

Exploring the genomes of *Phyllosticta*, a genus with multiple lifestyles

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Members of the fungal genus *Phyllosticta* can colonise a variety of plant hosts, including several *Citrus* species such as *Citrus sinensis* (orange), *Citrus limon* (lemon), and *Citrus maxima* (pomelo). Some *Phyllosticta* species have the capacity to cause disease, such as Citrus Black Spot, while others have only been observed as endophytes. Thus far, genomic differences underlying lifestyle adaptations of *Phyllosticta* species have not yet been studied. Furthermore, the lifestyle of *Phyllosticta citrichinaensis* is ambiguous, as it has been described as a weak pathogen but Koch's postulates may not have been established and the presence of this species was never reported to cause any crop or economic losses. Here, we examined the genomic differences between pathogenic and endophytic *Phyllosticta* spp. colonising *Citrus* and specifically aimed to elucidate the lifestyle of *Phyllosticta citrichinaensis*. We found several genomic differences between species of different lifestyles, including groups of genes that were only present in pathogens or endophytes. We also observed that species, based on their carbohydrate active enzymes, group independent of their phylogenetic association, and this clustering correlated with trophy prediction. *Phyllosticta citrichinaensis* shows an intermediate lifestyle, sharing genomic and phenotypic attributes of both pathogens and endophytes. We thus present the first genomic comparison of multiple citrus-colonising pathogens and endophytes of the genus *Phyllosticta*, and therefore provide the basis for further comparative studies into the lifestyle adaptations within this genus.

Screening for PFAS tolerance in white-rot fungi

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Per- and polyfluorinated substances (PFAS) are widespread, bioaccumulative compounds that are widely used in industry and daily life (*e.g*. aqueous firefighting foams, non-stick coatings). Because of the strength of the carbon-fluorine bonds in these compounds, PFAS cannot be removed from soil or wastewater using conventional methods. Recent research suggests oxidative mechanism of degradation and points toward white-rot fungi, known for their vast arsenal of oxidative enzymes.

In our study, we screened 72 strains of white rot fungi in order to evaluate their tolerance to high PFAS concentrations and ability to utilise PFAS as a carbon source. 83.3% of selected strains were able to grow in the presence of PFAS, with no evident inhibitory patterns, and in some cases only a subtle increase of growth on supplemented plates. In order to find a threshold value of the compound that would alter fungal growth in a more obvious way, we cultured the most prominent strains with increased concentration of PFAS. For one of the tested compounds, growth was highly inhibited in almost all of the strains.

Root-associated fungi and tree nutritional health

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Belowground fungal diversity has an influential effect on above ground in forest ecosystems. Changes in root-associated fungal communities are known to correlate with critical nutritional imbalances in the host trees as well as higher pest and pathogen susceptibility. This balance is fundamental to forest health yet there is limited literature on the matter. Building on previous research focusing on ectomycorrhizal communities, this study aims to identify root-associated fungi (*e.g.*, mycorrhizal fungi, pathogens, endophytes, and saprotrophs) present in the roots of beech, pine, spruce, and oak trees in 137 forest sites across 19 countries in Europe and the UK. Using bioinformatics and community statistics, we have generated a list of root-associated fungi of these dominant trees. In this study, we will explore the links between the proportions of these different fungal guilds, the abundance of certain fungi and tree mineral nutrition and forest condition. Nitrogen deposition, tree mineral nutrition, host root density and host species are expected to have the most significant link to presence of fungal species and guilds. We also hypothesize that sites with less ectomycorrhizal fungi diversity and poor environmental conditions (*e.g.*, high N deposition) will have higher presence of pathogens, endophytes and saprotrophs. Our findings on the links between community diversity of root-associated fungi and tree host and forest environmental factors will be used to help predict future forest conditions.

Molecular taxonomy of *Sporendonema*

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Sporendonema casei was described for the red mold on cheese, which is a consistent niche for this species. Additionally, *Sphaerosporium* (*Sph*.) *equinum* has been reported exclusively from cheese. Recently, species were reported also from sediment soil (*S. casei*), skin of bat wings (*Sph*. *equinum*) and insect pupa (*Sph. equinum*). Multilocus data analysis has shown that these two species are molecularly close to the genus *Arachniotus* and are classified in *Gymnoascaceae*, *Onygenales*. The present study aims to define borders of the genera *Sporendonema* and *Sphaerosporium*, as well as the nearest neighbor, *Arachniotus*. An additional cheese-associated fungus in *Hormiscium* was also included in the analyses.

Strains were evaluated in terms of colony morphology, microscopy, salt tolerance, growth rate at different temperatures, casein degradation and multilocus phylogeny with ITS, LSU and *TUB* locus sequences. Morphology and physiology test results did not reveal any specific characteristics to define genera. Phylogenetic analyses showed that the four generic names should be reduced to synonymy under *Sporendonema*. Two isolates of *Sph. equinum* isolated from substrates other than cheese were found to be phylogenetically and morphologically deviant and were introduced as a new species, *Sporendonema isthmoides*.

These fungi represent halophilic, psychrophilic, and xerotolerant members of the *Gymnoascaceae*. Conidial morphology, lipolytic ability, casein degradation and maximum growth temperature are variable between species but insufficient for accommodation in different genera. The individual species can be recognised by rDNA ITS as a primary barcode.

Detangling the mystery of the intriguing odor of dermatophytes

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Fungi are specialists in the degradation of hard to decompose materials. As an example, we can name the dermatophytes, filamentous fungi from the *Arthrodermataceae* family. During their evolution, dermatophytes acquired sophisticated mechanisms for utilisation of keratin, the main building block of skin, nails, claws, fur, and feathers of vertebrates. This ability accompanied by the capacity to grow at higher temperatures and to mask themselves from the host immune system makes them the most common fungal skin pathogens of humans, pets, farm animals as well as wild animals. During their growth, dermatophytes produce a whole variety of secondary metabolites into the cultivation media. On top of that, the dermatophytes emit a very strong and specific odor, composed of volatile organic compounds (VOCs). Spectra of fungal VOCs act as a potential tool for chemotaxonomy of and species determination. Additionally, the single compounds often pose strong cytotoxic and antimicrobial effects and therefore may play an important and to this day not considered role in pathogenesis. In our study, the GC-MS was used to measure VOCs of more than 14 taxonomical units of dermatophytes and in total; more than one hundred compounds were found. Preliminary results show that the chemotaxonomy based on VOCs spectra does not match phylogenetic taxonomy, which was also analysed as a part of this project. Therefore, VOCs spectra are not a reliable diagnostic tool for dermatophytes.

However, some of the individual compounds are not only found produced by fungi for the very first time. They even pose interesting potential biological activity, which is in the future going to be tested by bioassays and then tell us more about the role of VOCs in the pathogenesis of dermatophytes.

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Evolution-driven discovery of new bioactive fungal molecules

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Among other actions, finding new antimicrobials is key to treat infections caused by multi-resistant pathogens and at the same time better manage the emergence of resistance. Around 40% of the developed therapeutics drugs approved by the US Food and Drug Administration (FDA) are derived from natural molecules produced by plants, bacteria or fungi, showing these organisms are a unique resource of bioactive molecules. Recent genomic studies have revealed the great and largely unexplored biosynthetic potential of fungi to produce such molecules, and gaining access to this hidden diversity in a rational manner requires new approaches. In the present study, we focus on lichen mycobiont genomes, which can be hardly manipulated in the laboratory but have been used for centuries in traditional medicine and show a unique potential for the production of polyketide compounds. Using this lichen-associated fungal novelty as a starting point, we have employed a combined evolution-guided dereplication and comparative genomics strategy to identify novel polyketide biosynthetic pathways that could yield previously unreported molecules. Functional characterisation of the selected biosynthetic pathway using heterologous expression in *Aspergillus oryzae* revealed that the given pathway was linked to the production of naphthalenone compounds in fungi.

Endohyphal bacteria and metabolic capacities of *Mucoromycota* fungi

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Mucoromycota fungi are one of the most ancient groups of land fungi, with the majority being plant material-related soil saprotrophs. They form a metabolically and genetically diverse group of organisms able to use a plethora of carbon sources including organic polymers and to produce different types of secondary metabolites. Additionally, some lineages of *Mucoromycota* are known to harbor endohyphal bacteria (EHB). Through the combination of genomic and phenotypic methods the influence of these bacteria on the versatility of *Mucoromycota* fungi was tested. A symbiotic strain of *Paenibacillus* sp. seems to expand carbon assimilation capabilities of its host *Thamnidium elegans*, as the EHB positive strains efficiently degrade carboxylic acids and gelatin. Moreover, the presence of a *Paraburkholderia* sp. within the hyphae of an *Umbelopsis* sp., positively affects fungal capacity to degrade petroleum derivatives and polycyclic aromatic hydrocarbons, such as anthracene. Altogether, this research provides further evidence that endohyphal bacteria complement enzymatic capabilities of *Mucoromycota* representatives and alter their metabolism. However, metabolic pathways of these interactions remain largely unknown.

Coprophilous fungi: a source of bioactive secondary metabolites

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Coprophilous fungi are associated with the dung of animals. Herbivorous dung represents a rich medium for fungal growth and is a potential home to a large group of saprotrophic fungi. However, the biodiversity present in dung has been poorly studied as compared to other substrates, such as soil and plants. Moreover, coprophilous fungi are a potential reservoir of bioactive secondary metabolites. It has been postulated that they produce such compounds as defense against other microoorganisms and animals in the dung, resulting in enhanced ecological fitness of the producer strains in this highly competitive habitat (Bills *et al*., 2013). My DFG-funded project focuses on the exploitation of biological and chemical diversity of this group of fungi. We will focus in particular on strains of the order *Sordariales*, which are frequent in dung and known to be versatile secondary metabolite producers (Charria-Girón *et al.*, 2022). Most strains that we are about to study will be isolated from Germany, but we also collaborate with international partners in the course of an AvH Research Hub project with the University of Yaounde I (Cameroon) and in an ongoing H2020-MSCA-RISE project with partners from several European, Asian and African countries.

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Fungal microbiome of spruce bark beetle *Ips typographus* throughout its life cycle

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Bark beetles are an integral part of temperate forest ecosystems. However, they can also cause severe outbreaks as a result of global climate change, insufficient forest management and international trade. Bark beetles are associated with various microorganisms. These microorganisms facilitate beetles' development inside plant tissues as they detoxify them and provide beetles nutritional sources that are otherwise hardly accessible, *e.g.* nitrogenous substances.

Ips typographus is the most serious pest of European spruce forests. This beetle is known to be associated with filamentous fungi *Endoconidiophora polonica* and *Ophiostoma bicolor*, which help beetle detoxify spruce phloem; however, little is known about its whole microbiome. Our research is focused on description of bacterial and fungal taxonomic and functional diversity associated with *I. typographus* throughout its life cycle during spring and summer generation. We combine cultivation technic with metabarcode approach, metatranscriptome and genome sequencing. Here, we present our results of *I. typographus* fungal community structure revealed by metabarcode analysis and the first insights into the functional analysis of the dominant fungal symbionts derived from the genome sequencing.

The core microbiome of *I. typographus* remains stable throughout its life cycle and generations. However, the proportions of individual microorganisms significantly differ between generations, probably in response to actual environmental conditions. The fungal microbiome is dominated by saccharomycetous yeasts. The most common yeast species are *Wickerhamomyces bisporus*, *Kuraishia capsulata*, *K. molischiana*, *Nakazawaea ambrosiae*, *Yamadazyma* sp. and *Cyberlindnera* sp. Genome sequencing revealed that these yeasts have similar genetic potential. All these yeasts are able to degrade lignocellulose, and synthetise all essential amino acids and vitamin B6, which suggests their role in *I. typographus* nourishment. We detected both filamentous fungi *Endoconidiophora polonica* and *Ophiostoma bicolor* in the spring generation; however, in the summer generation, *E. polonica* was almost completely substituted by *O. bicolor*. These filamentous fungi were detected in very low abundances in uninfested phloem and parental adults but their abundances increase and finally they dominate in infested phloem at the end of *I. typographus* life cycle. To conclude, we propose that even though proportional taxonomic composition of *I. typographus* microbiome alternates between generations, the functional composition remains stable.

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