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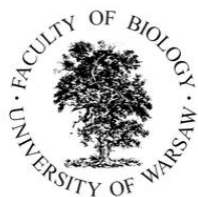
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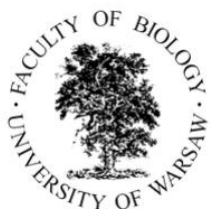
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Biology and Ecology of Fungi



Effectiveness of various methods of isolation of mycorrhizal fungal DNA from root samples

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Keywords: mycorrhizae, isolation, metabarcoding

I am a student of bioinformatics & biotechnology fascinated by the ways modern computational tools can aid researchers in their pursuit of knowledge.

Mycorrhizal fungi's role is in supplying plants with water, minerals & more. The vast majority of vascular plants cooperate with fungi. The specific nature of these relations remains hidden underground. To study them, new techniques must arise. A promising solution dwells in metabarcoding [2]. Databases of applicable sequences labelled with identifiers for taxa (with other, reliable methods) already exist [3]; many more are underway. As a recipient of the Best Student Grant I aim to study these techniques and eventually create a standardised methodology for such assays. For a lack of such standards renders these ingenious studies uncomparable to other studies.. So do many other factors (of which three were the concern of my current study).

Current stage of my research compares different methods of DNA isolation from root samples. Three independent variables: soil composition [4], isolation method & time interval between sample collection and DNA isolation. Two dependent variables monitored were: efficiency & unwanted contaminant levels.

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Worldwide taxonomy and phylogeography of a nivicolous myxomycete: striking contrasts between Northern and Southern Hemisphere

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Keywords: Amoebozoa, biogeography, long-distance dispersal, SSU, EF1A, nivicolous species

Nivicolous myxomycetes are an ecological group of fungus-like organisms associated with long-lasting snow cover. Their global diversity and biogeography remains poorly investigated. To advance it, we studied intraspecific diversity and phylogeography of a nivicolous species, *Didymium nivicola* Meyl. Based on an exhaustive range-wide sampling, we assessed its genetic diversity using two molecular markers (SSU and EF1A), and morphological variability using twelve preselected taxonomic characters. Our results revealed that: (1) two species can be delimited: *D. nivicola* with worldwide distribution, and newly recognized *D. pseudonivicola* Janik, A. Ronikier & Lado, which occurs only in the austral Andes, (2) two main parts of the *D. nivicola* range display contrasting phylogeographical patterns with high genetic diversity in the Southern Hemisphere (South America; 17 SSU and 12 EF1A variants) and genetic uniformity across the Northern Hemispheric continents (Europe, Asia, North America; one genotype), (3) an isolated population of *D. nivicola* from the Canary Islands (Africa) forms a distinct genetic lineage. We propose that the current global range of *D. nivicola* likely originated from an ancestral centre of origin in South America from which it spread to the Northern Hemisphere by at least two independent long-distance dispersal events. Subsequent long-distance dispersal within the Northern Hemispheric continents might be associated with a shift to asexual mode of reproduction.



Talk to the knowledge of mycodyversity in different forest associations on Shar Planina in RN Macedonia

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Keywords: Shar Planina, Macrofungi, Macedonian mycobiota

Shar Planina is the largest mountain massif in Macedonia that occupies an area of 1600 km². This massif is characterized with diverse geology, geomorphology and many glacial lakes, with continental, and alpine climate, making it very rich in biodiversity, which is very important from the conservation point of view (Melovski et al. 2013) and therefore it was recently proclaimed as National Park (2021). Data on fungal diversity from this mountain massif is scarce; there are only two papers with a list of species of fungi in certain areas of Shar Planina (Nastov et al. 1996, Karadelev et al. 2002). In order to provide better knowledge on fungal diversity, during the autumn season 2021 mycological forays in several forest types at different localities were performed. Material was collected from spruce forests (Jelak, Popova Shapka), beech forests (Jazince, Lisec, Gajre, Dobroste), and oak forests (Rogacevo, Jazince). A total of 80 species were identified, mainly from Basidiomycota (77), and most of them belong to the orders Agaricales, Russulales and Polyporales. During this season only 3 species of Ascomycota were identified. A total of 58 species were found in deciduous forests and 27 in coniferous forests. Some of the species can be underlined as rare, such as *Hericium coralloides* as EN on the Macedonian Red List of Fungi, and *Hohebuehelia petaloides* which represents a second finding for the country.

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How does soil pollution shape fungal communities?

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Keywords: soil pollution, mycoremediation, soil fungal communities

Fungi can be found in nearly all ecosystems. Some of them can even survive in harsh, anthropogenically transformed environments, such as post-industrial soils. In order to verify how the soil fungal diversity may be changed by pollution, we collected samples from 28 post-industrial sites with various degrees of contamination, ranging from non-polluted to heavy-polluted. Each soil sample was characterized in terms of concentration of heavy metals and organic petroleum derivatives. To identify soil fungal communities, fungal ITS2 amplicons were sequenced for each sample using the Illumina MiSeq platform.

The highest taxon richness and evenness were observed, as expected, in the non-polluted sites, and lower numbers of taxa were identified in multi-polluted soils. The presence of monocyclic aromatic hydrocarbons, gasoline, and mineral oil were determined as the factors driving the differences in the mycobiome. Additionally, our culture-based selection experiment suggests that it is long-term soil contamination that shapes the community, rather than the temporary addition of pollutants. Isolation of fungal communities on media with and without addition of pollutants allowed us also to delimit two main groups of fungi growing on polluted substrates – generalists able to live in the presence of pollution, and specialists adapted to use oil derivatives as a sole source of energy. The representatives of this second group are of particular interest in bioremediation.



Diversity of Icelandic Oomycota

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Keywords: diversity, taxonomy, Oomycota, *Phytophthora*, *Pythium*

Oomycetes, fungus-like protists of the kingdom Stramenopila that also includes diatoms and sea-weeds, were collected in July 2021 from various natural environments of Iceland. Substrates included plant debris, meadow and forest soil, pond water, and lake sediments. From this, 200 isolates were acquired. Based on morphological features and phylogenetic analyses of nuclear (ITS) and mitochondrial (cox1, cox2) loci, six oomycete genera, namely *Globisporangium*, *Halophytophthora*, *Phytophthora*, *Phytopythium*, *Pythium*, and *Saprolegnia* were identified. *Globisporangium attrantheridium* was the most abundant species obtained, followed by *G. heterothallicum*. *Pythium* sensu stricto isolates were located in clade B and identified as *Py. dissotocum* and *Py. oopapillum*. *Saprolegnia* isolates were grouped with *S. ferax* based on ITS and cox1 regions; however, the cox2 locus separated them into a distinct group. *Phytophthora* isolates could be assigned to *P. inundata*. The presence of *Halophytophthora* is noteworthy, as the genus has so far been mainly reported from tropical regions. *Halophytophthora* sequences were identical with previously deposited sequences of an undescribed species. A new species of *Phytopythium* was identified based on morphology and a clear separation from known taxa in all phylogenetic trees. Our findings highlight that also in the arctic environment oomycetes are species-rich and new species, potentially adapted to the harsh climatic conditions at high latitudes can be found.



Diversity of Hypogeous Genus *Hymenogaster sensu lato* in the Republic of North Macedonia

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Keywords: revision, morphological species concept, diversity, hypogeous fungi

Hymenogaster is an ectomycorrhizal genus producing sequestrate, hypogeous fruiting bodies; widespread and diverse, with around 100 species described; yet, recent molecular phylogenetic analyses have shown it is a polyphyletic genus. Until 2019, nine species had been recognized in RN Macedonia: *H. arenarius* Tul. & C. Tul., *H. bulliardii* Vittad., *H. citrinus* Vittad., *H. griseus* Vittad., *H. luteus* Vittad., *H. lycoperdineus* Vittad., *H. olivaceus* Vittad., *H. populetorum* Tul. & C. Tul., and *H. rehsteineri* Bucholtz [1-3]. Revision of deposited collections along with examination of fresh material has been conducted. The material has been identified by following the morphological species concept, and the identification key by Montecchi and Sarasini [4]. The revision has demonstrated that the collection formerly identified as *H. arenarius* resembles *H. niveus* Vittad., while the collection named *H. bulliardii* does not belong to the concept of this species, and it is noted as *Hymenogaster* sp. Beside *H. niveus*, two more species have been identified for the first time in RN Macedonia, *H. hessei* Soehner and *H. vulgaris* Tul. & C. Tul. In summary, 10 species of *Hymenogaster sensu lato* have been documented to occur in RN Macedonia. Nevertheless, research of combined morphological and molecular phylogenetic analyses should be accomplished to clarify the genus diversity in the country.

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Biotechnology of Fungi



Degradation of Oil Derivatives by representatives of Mucoromycota

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Keywords: bioremediation, hydrocarbon degradation, Mucoromycota, endohyphal bacteria

Oil derivatives are common soil pollutants. They have a negative impact on the environment, as they change the physical and chemical properties of soil ecosystems. Many of them, particularly polyaromatic hydrocarbons (PAHs), are also highly toxic. Bacteria and fungi are promising candidates for bioremediation, as some are able to degrade these stable, long-living compounds. In contrast to bacteria, not much is known about the fungal metabolic pathways used for these processes.

We investigated the compounds present after culturing two strains of fungi on mineral media with three different carbon sources, which represent the main chemical groups within oil derivatives: PAHs (anthracene), aliphatics (undecane), and alicyclics (cyclohexane). The strains used belong to the genera *Umbelopsis* and *Rhizopus*. Both harbor endohyphal bacteria, so we used the original strains as well as cultures that had been cured of bacteria with ciprofloxacin. Gas chromatography coupled with mass spectrometry was used to analyze the compounds present. The saturated compounds (undecane and cyclohexane) seem to be taken up and then follow the previously-suggested β -oxidation route. The results from anthracene samples, however, show a large number of different compounds produced. These chemicals also differ between species and whether bacteria were present or not. We attempted to reconstruct the anthracene degradation pathways from these results.



The impact of an innovative fertilizer biostimulator on the enzymatic activity and biodiversity of soil microorganisms under cauliflower cultivation

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Keywords: fertilizer, biostimulator, cauliflower, *Trichoderma*

The intensive development of agriculture has led to the misuse of artificial fertilizers, which entails numerous threats to the environment and human health. This situation contributed to the search for alternative bio-fertilizer prototypes supporting the development and yielding of plants. The effectiveness of fertilizing products resulting from the presence of an organic substance, additionally enriched with beneficial microorganisms, allows them to be used in agriculture, and additionally brings many environmental, health, and economic benefits.

The aim of the study was to evaluate the effect of fertilization with an innovative fertilizer biostimulator based on lignocellulosic material inoculated with *Trichoderma* spp., *Bacillus* sp., *Pseudomonas* sp. and *Streptomyces* sp. on changes in the activity and population of soil microorganisms and the yield of cauliflower plants. The greenhouse experiment was carried out with the use of the Momentum variety with 19 fertilization variants, on two types of soil.

The obtained results showed that the application of all innovative biostimulants in cauliflower cultivation had a positive effect on its yielding, and caused an increase in the activity of dehydrogenases and the BIF index. In variants enriched with *Trichoderma* spp. strains, an increase in the percentage of Actinobacteriota was noted.

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***Neosartorya* spp. - the hidden threat to agriculture and food production**

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Keywords: heat resistance, metabolic differences, plant extracts, food preservatives

Fungi belonging to the *Neosartorya* genus are very often heat-resistant and able to withstand standard product treatments and, in consequence, are able to cause spoilage. They are naturally present in soil and plant residues and characterized by ascospore-forming structures.

We hypothesize that differences in metabolic, morphological, and genetic properties between strains of *Neosartorya* genus have a role in shaping their resistance to preservatives, chemicals and natural plant extracts and that those differences contribute to overall resistance of these fungi to environmental factors. It is of utmost importance to investigate isolates of varying sensitivity and create novel, ecologically sound methods of agriculture protection. Using Sanger sequencing, inhibition tests, and statistical analysis, we were able to select isolates presenting different responses to plant extracts, sugar and pH combinations, and preservatives, permitting the selection of fungi for future study of fungal phenotypical features and their chemical sensitivity.

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Mucoromycota as potential source of biosurfactants

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Keywords: Mucoromycota, biosurfactants, review

Surfactants are compounds that reduce surface and interfacial tension. Wide usage of surfactants in industry and recent worldwide attention to environmental safety creates a demand for low toxicity, biodegradable alternatives. Biosurfactants are amphiphilic compounds produced by diverse microorganisms, mainly bacteria. Fungi are also well-known producers of such molecules. Several studies on biosurfactant synthesis in phylum Basidiomycota and Ascomycota were recently published. Although Mucoromycota representatives are rapidly growing and are easy to handle in biotechnological processes, they still remain understudied in terms of biosurfactant production. The aim of this study is to review available scientific data regarding biosurfactants production by Mucoromycota and evaluate their potential for further biotechnological applications.



Diversity of the fungal community on the dry aged beef

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Keywords: diversity, proteolysis, psychrotolerant *Mucoraceae*

Mucoraceae is a family of early diverging fungi usually recognised as environmental generalists often isolated from soil samples. However, there seems to be some specialisation among their members. For example, those closely related to *Mucor flavus* are well adapted to grow in low temperatures. One of the environments in which those psychrotolerant fungi are present is dry aged beef. While sometimes they were described as agents of food spoilage, recent research may prove them to have a positive influence on the meat ageing process, improving the taste of the final product. As little was known about overall fungal communities developing on dry aged beef, We aimed to characterise it using classical microbiological techniques and next-generation sequencing-based methods. Both methods provided similar results, however there were some discrepancies. 19 strains of hyphal fungi were isolated from 9 samples using traditional methods, majority of them belonging to the *Mucor flavus* species complex which corresponded with metabarcoding data from selected samples. Both of these closely related taxa were previously described from meat samples and seem to be characteristic for the dry aged beef communities.

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Fungi in Interactions



Microorganisms associated with greater mouse-eared bat (*Myotis myotis*)

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Keywords: *Myotis myotis*, microorganisms, underground ecosystems

Greater mouse-eared bats (*Myotis myotis*) might be a reservoir of microorganisms [1,2]. Thus, the aim of the study was to review the available literature on *M. myotis* living in underground sites as a microbial reservoir. Fungi isolated under the clusters of hibernating *M. myotis* in the Nietoperek Bat Reserve (NBR) were: *Absidia glauca*, *Aspergillus fumigatus*, *A. tubingensis*, *Mortierella polycephala*, *Naganishia diffluens*, and *Rhodotorula mucilaginosa* [1]. On the other hand, 17 fungal species were obtained from the surface of wing membranes and *Penicillium chrysogenum* was the most dominant. Additionally, *Pseudogymnoascus destructans* – the causative agent of the White Nose Syndrome – was found repeatedly in *M. myotis* around the world, including Poland, France, Czech Republic, or Slovakia. Overall, 8 species of airborne bacteria were isolated under the clusters of hibernating *M. myotis* in the NBR, and Actinobacteria were dominant in the study. *M. myotis* are natural hosts for viruses of the Lyssavirus genus [3]. EBLV-1 is the most common European bat lyssavirus. Alphacoronavirus, Mamastrovirus, as well as adenoviruses were also recorded in *M. myotis* colonies [4]. *M. myotis* can become a reservoir of some viruses from the Paramyxoviridae, Parvoviridae, and Caliciviridae families. In conclusion, *M. myotis* is a reservoir of bacteria, viruses, and fungi in underground sites, including those that pose a potential threat to mammals.

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Insight into ectomycorrhizal fungi communities of *Dryas octopetala*. What shapes them?

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Keywords: Alpine environment, climate change, Carpathians, ECM, tundra

Ectomycorrhizal (ECM) partnership between plants and fungi proves most beneficial in harsh environments such as alpine tundra, where drought, short vegetation period and low levels of accessible nutrients are limiting plant growth. Numerous factors can affect fungal communities interacting with plants, from host specificity, limiting potential fungal partners, to extreme environmental conditions promoting dominance of few adapted taxa. By comparing ECM symbiont communities of arctic alpine shrub *Dryas octopetala* in contrasting populations, with the aid of climatic data, we tried to shed light on this problem.

Overall 330 ECM root tips, from typical (4) and relic (2) *D. octopetala* vegetation sites located in Carpathians, were identified to species or genus level based on nrDNA sequences. All fungal communities were characterised by high species richness and dominance of a few genera such as *Tomentella*, *Cenococcum* and *Sebacina* (mostly ecological generalists). Basidiomycetes occurred more frequently in alpine zones at the expense of ascomycetes. We concluded that investigated communities have high adaptive potential and are mainly shaped by abiotic factors. However, we also observed a notable lack of the genera *Russula* and *Lactarius* in the root samples, despite their presence on investigated sites. This may indicate some level of ecological specificity for certain fungi, in the case of the Carpathian populations of *D. octopetala*.



Appreciated Laboulbeniales and neglected *Aegeritella* - ectoparasitic fungi of European ants

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Keywords: parasites, insects, Formicidae, interactions

The ecological group of ectoparasitic fungi of European ants includes genera: *Rickia*, *Laboulbenia* and *Aegeritella*. While the first two are closely related and belong to Laboulbeniales (Ascomycota), *Aegeritella* belongs to Tremellales (Basidiomycota). Those fungi also differ in morphology. Laboulbeniales produce multicellular units - thalli, that form perithecia where ascospores are produced. Meanwhile, *Aegeritella* grows in yeast-like colonies (bulbils) forming reproductive phialide-like structures. Apart from those differences, all those fungi do not penetrate the cuticle of ants and were categorised in previous studies as “mild parasites” with negligible effect on their host. However, at least in the case of Laboulbeniales, recent research demonstrates that those ectoparasites significantly change the lifespan of infected individuals, influencing the body mass of the workers or their behaviour. With *Aegeritella*, apart from a few anecdote examples, we still do not know how it changes the behaviour and fitness of its hosts. Research on *Aegeritella* refers to the geographical occurrence, distribution of the bulbils on the body of the host, and their prevalence.

In this presentation, we would like to point out the gaps in the knowledge in the biology of *Aegeritella* compared to Laboulbeniales and propose research that could be performed to fill those gaps. We hope that such a review will encourage mycologists to work more on ectoparasitic fungi, especially on those previously neglected.



Mycobiota of *Formica polychetena* - in a search of undiscovered symbiosis

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Keywords: fungal interactions, insects, mycobiota

In Europe, previous studies focused mostly on ant-parasitic species, neglecting other fungi present in the ants' surroundings. The purpose of this project was to investigate the yet unknown mycobiota of *Formica polychetena* ants in order to distinguish their symbionts. *F. polychetena* is a common species in temperate forests, strongly affecting the surrounding ecosystem. These ants live in mounds which are composed mostly of organic matter and are rich in antifungal substances. Such specific properties create a unique environment within the forest litter. In the set of studies we analyzed fungal communities: on the cadavers of ants, in the ants' infrabuccal pockets (in which spores are collected during ants eating and grooming), and in the mound material. Our results suggest that such fungal communities differ from those in the surrounding environment, and that genera: *Penicillium*, *Cladosporium*, *Entomortierella*, *Trichoderma*, *Schwanniomyces*, and *Absidia* are common in the ants' surroundings. The effect of such fungi on ants remains unknown. While entomopathogenic properties were noted before only for a few representatives of those taxa (mostly of *Cladosporium*), other fungal representatives can affect ants positively (e.g. nutritional value of *Entomortierella*). Interestingly, *Entomortierella*, *Absidia*, *Schwanniomyces* and *Penicillium* have been also previously isolated from other ant species, which raises a question of these taxa adaptation to the ant-made environment in general.



Correlations between fungal trophic modes in healthy and unhealthy strawberry samples

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Keywords: fungal diversity, fungal microbiome, organic agriculture

Plants and microorganisms that live on the surface and inside plant organs have developed complex networks of relationships between them. Fungal microorganisms, among others, are important members of the environment that shape plant fitness and are vital to the ecology. Beside the taxonomy, they can be assigned to trophic modes (pathotroph, symbiotroph, saprotroph and mixed) that play different roles in the community. We aimed to uncover correlations between fungal trophic modes present in bulk soil, rhizosphere, roots and shoots that we collected in healthy and unhealthy organic strawberry plantations. We isolated the DNA from environmental samples and sequenced ITS1 fragments of fungal DNA with Illumina MiSeq sequencer and then assigned the taxonomy with the UNITE 8.3 database. We then used FunGuild and FungalTraits databases in RStudio to assign trophic modes and calculated Kendall correlation ranks and statistical significance ($p\text{-value} < 0.05$). The analysis revealed that fungal trophic modes in healthy plant samples do not develop significant correlations, whereas in unhealthy plant samples, negative correlations between some of the trophic modes are visible.

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Biochemical diversity of lichen secondary metabolites as an adaptation to harsh environmental conditions

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Keywords: lichens, secondary metabolites, microbiome, extremophiles

Lichens (lichenized fungi) are a group of pioneer symbiotic organisms of a unique morphology, physiology, microbiome, and metabolism. Their main secondary metabolites, which are terpenes, phenol acids derivatives, anthraquinones and pulvinic acids, show wide range of biological activities: antioxidative, antibacterial, antifungal, antiviral, and cytotoxic properties, ability to absorb UV-A and UV-B light, and a role in tolerance for high heavy metal concentrations. Possible mechanisms of their activity are discussed. It is shown that the lichen secondary metabolites are crucial for a pioneer character and adaptation success in environments hard to colonise for other organisms, as well as their complex structure containing an opulent microbiome. Current and potential uses of lichen secondary metabolites are defined: in industry, pharmacology, medicine, bioidentification, bioremediation, and even astrobiology.



Pathogenic Fungi



Characteristics of yeast colonization in patients with exacerbations of COPD - preliminary studies

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Keywords: COPD, exacerbation, yeast

Patients with chronic obstructive pulmonary disease (COPD) are more susceptible to fungal colonization or infection. Recently, awareness of the importance of fungal colonization in the patients with COPD was rising. The aim of the study was to determine the level of fungal colonization in patients during exacerbations of COPD. The material for the study was oral swab, spontaneous sputum samples, and stool samples, collected from 15 patients with COPD exacerbation, GOLD stage 4, none of them had been treated with antifungal agents. Mycological cultures were carried out on Sabouraud medium with chloramphenicol (GRASO Biotech). The obtained yeast colonies were counted and biochemically identified using the Integral System Yeast Plus (Liofilchem®), which also allows for a qualitative assessment of drug susceptibility to 10 antimycotics. The presence of yeast was found in 15 patients, including 2 patients with yeast isolated from all analyzed materials. A total of 27 yeast isolates belonging to 6 species were obtained. The dominant species was *Candida tropicalis*. 55% of the isolated yeasts were sensitive to all assessed antimycotics, the remaining strains showed intermediate resistance to single drugs only. The level of yeast colonization in COPD patients is very high. The same species was found in different materials in the same patients, which may indicate multifocal colonization. All strains were sensitive to most antimycotics examined.



The effect of light conditions on the metabolic activity of selected *Fusarium temperatum* strains

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Keywords: *Fusarium temperatum*, mycotoxins, pigments, light conditions

Fusarium temperatum Scaufl. & Munaut is one of the causative agents of maize ear rot increasingly commonly recorded in the temperate climate zone. The harmfulness of the disease results not only from the decrease in the quantity and quality of the yield, but also from the pathogen's ability to biosynthesize secondary metabolites, including those with toxic properties.

The aim of the study was to assess the ability of the selected *F. temperatum* strains to biosynthesize secondary metabolites *in vitro*. The research material consisted of single-spore *F. temperatum* strains obtained from infected corn cobs. The fungal cultures were grown in Petri dishes with Rice Broth medium under the following light conditions: white, red, blue, ultraviolet, and no light. Samples for spectrophotometric analyses were collected every 24 h, from the 4th to the 8th day of incubation.

The light conditions under investigation had a significant effect on the biosynthesis of secondary metabolites by *F. temperatum* strains. The highest content of metabolites was found in the extracts obtained from cultures incubated in no light conditions. In the obtained metabolite extracts, a significant increase in absorbance was noted at wavelengths from 480 to 510 nm. The studied *F. temperatum* population demonstrated high inter-individual variability in terms of the level of metabolite biosynthesis and the response of the strains to the applied light conditions.



Exploring the role of host chloroplast targeting non-classically secreted effector proteins in the development of rice blast disease

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Keywords: non-classically secreted, *Magnaporthe oryzae* effectors, chloroplast targeting

Plant pathogens partly modulate host immunity by secreting proteins around and into their host cells. Here, we premised the identification, and functional characterization of non-classically secreted effector candidates in the pathogenesis of the globally destructive rice blast fungus, *Pyricularia oryzae* (syn. *Magnaporthe oryzae*). This study aimed to deploy an in-house machine learning model to identify and functionally characterize non-classically secreted effector candidates in *M. oryzae* that exclusively target host chloroplast during pathogen-host interaction. We identified 55 proteins targeting host chloroplast. Results showed that GFP constructs of MoCHLO1,3, NED and 22 colocalized rice chloroplast protein RFP-Chcpn10 in planta. Our targeted gene deletion strains showed that MoCHLO3 and MoCHLO22 completely abolished the pathogenicity of the rice blast fungus. However, targeted gene deletion of other studied genes had no observable adverse effects on the virulence of *M. oryzae*. We speculated that chloroplast localization of MoCHLO3 and MoCHLO22 is likely essential for the pathogenicity of *M. oryzae*. Also, we demonstrated that the transient expression of MoCHLO3 without cTP attenuated the localization of GFP-MoCHLO3 to the chloroplast and triggered the accumulation of GFP-MoCHLO3 fluorescence signal in the pavement cells of epidermal tissues. No GFP signals were found on deletion of cTP from MoCHLO22. However, further research is required to go in depth.



Diversity of potentially pathogenic fungi associated with the grey seals *Halichoerus grypus* (Fabricius, 1791)

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Keywords: mycobiota, mycoses, pathogens, interactions, diversity

Grey seal *Halichoerus grypus* (Fabricius, 1791) is an endangered species of great importance for the ecosystem of the Baltic Sea, where seals are apex predators. Seal sanctuaries and wildlife rehabilitation centers accept individuals all year round. Chronic captivity stress and injuries make seals more susceptible to infections, making prevention and diagnostic testing key factors. However, there is little knowledge about the fungal pathogens of seals and other Phocidae. Most of the described seals' mycoses concerned the outer integuments. The goal of our project was to contribute to determining the fungal diversity associated with the skin and fur of *H. grypus*.

We analyzed 28 samples of fur in total (17 samples from 5 healthy captive seals and 11 samples from 11 wild pups). Fungi were isolated on 4% Sabouraud agar with chloramphenicol (4 weeks, 25 °C). Isolated strains were identified based on sequencing of ITS rDNA marker. 14 of ~35 isolated species are prototypical fungal pathogens or opportunistic pathogens. So far no infection caused by these fungi has been reported in grey seals, however, some are pathogens of other marine mammals, which makes them potentially dangerous also for *H. grypus*. Further knowledge of potential reservoirs of pathogens could enable better prevention and treatment in the future.



Humans & Fungi



From databases to compound producers - activity of polyketide macrolactone biosynthetic gene clusters in response to different stimuli in diverse ascomycetes

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Keywords: Toxins, Gene Clusters, stress response

Benzenediol macrolactones (BDLs) are hydrophobic fungal polyketides with potent bioactive properties (potential drug leads). Their role in nature frequently hinges on antimicrobial and/or antifungal action against broad groups of organisms, thus the basic structure has largely diversified as a result of the ancient but ongoing ‘fungal war’ for ecological niches. As for other secondary metabolites, lactone biosynthesis is often influenced by environmental signals indicating the presence of competitors (whether saprobic or pathogenic) or plant host tissue (plant pathogens).

Following our previous analysis of >1K publicly available fungal genomes and screening for BDL-like core polyketide synthase genes, we successfully identified, sequenced and annotated genomes of 17 new potential BDL producers amongst diverse filamentous fungi. Apart from *in silico* analysis of candidate clusters composition and phylogeny, we report on the influence of multiple chemical stimuli (azacitidine, cinnamic and ferulic acids, H₂O₂) on cluster activity. Both expressions of core biosynthetic genes and presence of major, known lactone compounds were characterised for 6 candidate strains (*Diaporthe toxica*, *Diaporthella corylina*, *Curvularia inaequalis*, *Curvularia affinis*, *Fusarium cerealis*, *Leucostoma cinctum*, *Penicillium sanguifluum* and *Talaromyces acaricola*).



Analysis of medicinal and nutritional properties of selected mushroom species from the genus *Hericium*

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Keywords: nutritional, medicinal, mushroom cultivation

The Genus *Hericium* belongs to the family of *Hericiaceae*, covering saprotrophic mushrooms found throughout the world. All species resemble small white icicles, growing either from branches or from clumps of various sizes. The most popular species of this genus is Lion's Mane (*Hericium erinaceus*) characterised by nootropic properties. Many other species belonging to this genus have not been investigated. This presentation shows the chosen results of studies conducted at the University of Agriculture in Cracow in collaboration with Jagiellonian University in Cracow, Poland. The chemical composition of three mushroom species were analysed: *H. erinaceus*, *H. americanum* and *H. coralloides*. These analyses were conducted in both fruiting bodies cultivated in a wood substrate, and mycelium grown in bioreactors. The results showed differences in chemical composition between species and sampling material (fruiting bodies and mycelium).

The work was supported by the Ministry of Education and Science project “Studenckie Koła Naukowe Tworzą Innowacje”, Poland, SKN/SP/496634/2021.



Novel benzoxaborole derivatives against virulence of *C. albicans*

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Keywords: antifungal activity, benzoxaborole, *Candida*

Fungal infections are a serious clinical problem on a global scale. Studies conducted over the past two decades have shown an increase in invasive fungal infections, especially those caused by *Candida* spp. Candidiasis is a comorbid disease – along with other diseases it is a lethal threat, responsible for 30-50% of mortality among patients. Especially today, patients suffering from coronavirus (COVID-19), treated with immunosuppressants such as dexamethasone or IL-6 inhibitors, are particularly at increased risk of systemic fungal infection (most dangerous for humans). Recent reports suggest that the available antifungal drugs are ineffective due to the emergence of new forms of resistance to *Candida* spp., toxicity related to the side effects of antimycotics, and interactions with other medicinal products. It is therefore a priority to find and test new antifungal substances, with particular emphasis on the lack of toxicity to the patient.

Based on the preliminary research carried out by the Biotechmed-3 project, benzoxaborole derivatives, with particular emphasis on the bis (5-fluoro benzoxaborole) derivative, seem to be a promising target due to their low toxicity to the VeroE6 cell line coupled with high antifungal activity, also against *Candida* biofilm. Our studies are focused on the analysis of flow cytometry and molecular biology, which will elucidate the mechanism of action of novel benzoxaborole derivatives against pathogenic *Candida*.



Ozonation of the mushroom substrate - an innovative and ecological technology

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Keywords: mushrooms, cultivation, edible, medicinal

Cultivation of mushrooms is an ecological and modern horticulture industry with great potential. More than one hundred species of mushrooms are grown all over the world, which are valuable and nutritious food for humans. Mushroom cultivation technologies are constantly developed, and the basic goal is to make them as energy-conserving and waste-free as possible. The aim of this presentation is to show the results of a project financed by the Ministry of Education and Science “Student science clubs are creating innovations” entitled “Innovative technologies in the cultivation of edible and medicinal mushrooms”. Within this project, ozonation of mushrooms substrate was tested for 7 species of gourmet mushrooms: *Pleurotus ostreatus*, *Pleurotus eryngii*, *Pleurotus citrinopileatus*, *Hericium erinaceus*, *Agrocybe aegerita*, *Flammulina velutipes* and *Pholiota adiposa*. The results show a great potential of this technology compared to the traditional methods of substrate sterilization. Experience has shown that ozonation of the substrate can replace standard sterilization methods. Mushroom fruiting bodies obtained from ozonated substrate do not differ in biological activity and total harvest compared to standard method of cultivation.

The work was supported by the Ministry of Education and Science project “Studenckie Koła Naukowe Tworzą Innowacje”, Poland, SKN/SP/496634/2021



Posters



Impact of long-term freezing on biodiversity of Mucoromycota strains isolated from soil samples

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Keywords: biodiversity, freezing, temperature, Mucoromycota, long-term storage

Culture-based analysis of fungal diversity is still one of the most commonly used methods for studying soil mycobiome. Nevertheless the analysis may take up many months. During that time the samples are often stored in -20°C . For a long time it was not considered an issue, because soil fungi of central Europe have to cope with annual temperature decreases, reaching -20°C . However, it was recently shown that cultures of some European, ectomycorrhizal fungi cannot survive four-hour exposure to -8°C . Similarly some Mucoromycota lose their viability during storage in 4°C for long periods of time (10-13 years). Consequently it was hypothesised that storing soil samples in -20°C for a long time, may impact the results of culture-based soil Mucoromycota biodiversity studies.

To test the hypothesis, 4 soil samples were taken. From two of them 24 Mucoromycota strains were isolated immediately. Two other samples were stored in -20°C for two years - then 25 Mucoromycota strains were isolated. All isolates were classified with an accuracy up to genus level using ITS marker and Blastn searches against the whole NCBI database. Biodiversity of isolates from frozen samples (Shannon-Wiener's index = 0.849) was significantly lower than of those isolated from „fresh” samples (Shannon-Wiener's index = 1.203) - mean p.value obtained from 1000 permutation tests equaled 0.047 (SD = 0.0031). Freezing samples in -20°C for 2 years probably decreases the biodiversity of isolated Mucoromycota strains.



Trinity - a review of three most destructive *Basidiomycota* fungi causing brown rot of wood

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Keywords: wood decaying fungi, brown rot, Basidiomycota, wooden buildings

The poster contains the most important information concerning three species of fungi, belonging to the Basidiomycota phylum, which cause brown decay of wood. This degradation, next to insects, is the most dangerous biological factor causing wood deterioration. The consequence of the decomposition is a significant loss of mechanical properties of this material. It is particularly important in relation to buildings, because of the structural functions. On the basis of literature, a brief characterization of the three most dangerous fungi species has been carried out: *Serpula lacrymans*, *Coniophora puteana* and *Fibroporia vaillantii*. A correlation between brown rot decay and loss of mechanical properties of wood was shown. The aim of this work was to compare the most important species of Basidiomycota fungi causing brown decay of wood in terms of optimal growth conditions and its negative effects on wooden buildings. On the basis of the comparison, *Serpula lacrymans* was found to be the most dangerous in terms of degradation rate and lowest moisture requirements. This fungus is the most common species in wooden buildings.



The ability of *Umbelopsis* representatives to produce polyunsaturated fatty acids

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Keywords: biodiesel, lipids, polyunsaturated fatty acids (PUFAs), *Umbelopsis isabellina*

Phylum Mucoromycota consists of ecologically and economically important fungi. They are efficient decomposers of organic matter in soil but they also may provide us with enzymes and metabolites, which are of use in various industry branches. A number of recent studies show that their ability to accumulate lipids is outstanding especially when grown on low-value substrates e. g. agricultural, food or glycerol waste. Currently, there is a growing interest in microbial oils, also called single cell oils (SCOs). However, the price of obtaining them on a large scale is still too high due to the low productivity of oleaginous organisms in relation to the high fermentation cost. Therefore, searching for rapidly growing organisms able to accumulate a high amount of lipids is of great importance. *Umbelopsis isabellina* (Mucoromycota) is one of such promising species. It is able to efficiently produce monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), such as e. g. γ -linolenic acid (GLA), which could be potentially used as precursors in 2nd generation biodiesel, diet supplements and pharmaceuticals production.



Prevalence and genetic basis of terbinafine resistance in *Trichophyton rubrum*, a cause of superficial mycoses

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Keywords: Dermatophytes, resistance, antimycotics, *Trichophyton*

Trichophyton rubrum is the most dominant cause of superficial mycoses (dermatophytosis) in humans in developed countries. A relatively wide range of antifungals of different chemical classes are available for the treatment of dermatophytosis, but the cellular targets of these drugs are relatively limited. Primary targets of these substances are various steps of ergosterol synthesis, thereby disrupting the cell membrane. The problem of recent years is the spread of resistance against terbinafine, which is currently one of the most widely used drugs. The molecular essence of the resistance lies in mutations of the gene responsible for production of the enzyme squalene epoxidase (SQLE), one of the key enzymes in ergosterol synthesis that terbinafine targets. The alarming increase in resistance is currently being described mainly in India and these resistances are spreading in European countries as well, but the situation in the Czech Republic has not been studied. The goal of the present project is to determine the prevalence of resistance in *T. rubrum* isolates from Czech patients. Another aim is to determine the prevalent molecular mechanisms of resistance, which will be achieved by sequencing the SQLE gene and analysis of mutations. Results of the project may have an impact in the treatment of dermatophytosis and will help map the incidence of resistance in the Czech Republic. Part of the project is also to understand the causes of resistance in species from clinical isolates.



Pleurotus ostreatus* - growth of the mycelium and nematicidal properties against *Caenorhabditis elegans

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Keywords: nematodes, oyster mushroom, toxins, mycelium growth

Oyster mushroom (*Pleurotus ostreatus*) is one of the many fungi with nematicidal properties. The main defense mechanism of the oyster mushroom is the production of a toxin (trans-2-decenoic acid) that paralyzes the nematode on contact. This toxin is produced only on the protuberances of the hyphae, which take the characteristic round shape (drop shape). After contact with the toxin, the nematode becomes paralyzed and provides an ideal source of nutrients for the mycelium that grows over the body of the dead nematode. This research presents the ability of two strains of *P. ostreatus* to produce hyphal knobs releasing toxic droplets and differences in the development of their mycelia.



How high do you need to hike to find *Lamproderma* species?

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Keywords: SDM, ENM, maximum entropy, myxomycetes, *Lamproderma*

For myxomycetes, with few exceptions, seasonal dynamics of fruiting is characteristic. Special conditions are required for nivicolous myxomycetes. They form sporocarps in spring when the snow melts, often on the remnants of herbaceous plant litter from the previous season. The territory of the Czech Republic is surrounded by middle-high mountains, which creates mild climatic conditions in the lowlands of the country. The study of myxomycetes in the above-mentioned territories was fragmentary and at different time periods. Considering this for further research, it is important to define the pattern of possible fruiting of these species.

The aim of my present study is to define the ecological niches for members of the genus *Lamproderma* in the region of Czech Republic and understand which factors limit their distribution.

Occurrence records of *L. arcyrioides*, *L. collumbinum*, *L. gulielmae*, *L. sauteri*, *L. scintillans* were obtained from literature. Data from the WorldClim archive and sub-canopy microclimate temperatures of European forests were used as climatic variables.

Using various methods of mathematical modelling (MaxEnt, BARTS and RF), the most probable fruiting sites of *Lamproderma* species were calculated. The results of conducted modelling showed that the species occurrences of this genus do not depend on altitude, but rather on macro climatic characteristics, such as: mean temperature of the wettest quarter and precipitation of the warmest quarter.



Catabolic fingerprinting and diversity of fungi in the degraded soil amended with phosphorus biofertilizer

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Keywords: metabolic fingerprinting, phosphorus biofertilizer, soil fungi, BIOLOG™ Plates

Soil fungi perform a number of essential tasks for the proper functioning of the soil ecosystem. The aim of the study was to determine the metabolic diversity of fungal communities in the degraded soil fertilized with phosphorus biofertilizer. The two-year field experiment included the following treatments: FC-optimal dose of fertilizer without microbial enrichment, FA100-optimal dose of fertilizer enriched with microorganisms, and FA60-microbiologically enriched dose reduced by 40%. Soil samples were collected on three dates: autumn 2018 (A18), summer 2019 (S19) and autumn 2019 (A19). The metabolic potential of the soil fungal communities was determined with the application of the BIOLOG™ FF Plates system. The principle component analysis (PCA) showed a clear clustering of treatments with respect to the soil sampling time. FA100 and FA60 treatments were characterized by the increased number of utilized carbon substrates (richness), Average Well Color Development (AWCD) values and Shannon diversity index (H).

The work was co-financed by The National Centre for Research and Development in the frame of the project BIOSTRATEG, contract number BIOSTRATEG3/347464/5/NCBR/2017.



***In Silico* Predictions of Ecological Plasticity Mediated by Protein Family Expansions in Early-Diverging Fungi**

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Keywords: protein family; early diverging fungi; cell wall; adaptasome

Early-diverging fungi (EDF) are ubiquitous and versatile. Their diversity is reflected in their genome sizes and complexity. For instance, multiple protein families have been reported to expand or disappear either in particular genomes or even whole lineages. The most commonly mentioned are CAZymes (carbohydrate-active enzymes), peptidases, and transporters that serve multiple biological roles connected to e.g., metabolism and nutrients intake. In order to study the link between ecology and its genomic underpinnings in a more comprehensive manner, we carried out a systematic *in silico* survey of protein family expansions and losses among EDF with diverse lifestyles. We found that 86 protein families are represented differently according to EDF ecological features (assessed by median count differences). Among these, there are 19 families of proteases, 43 CAZymes and 24 transporters. Some of these protein families have been recognized before as serine and metallopeptidases, cellulases, and other nutrition-related enzymes. Other clearly pronounced differences refer to cell wall remodelling and glycosylation. We hypothesize that these protein families altogether define the preliminary fungal adaptasome. However, our findings need experimental validation. Many of the above mentioned protein families have never been characterized in fungi, and are discussed in the light of fungal ecology for the first time.



Relationships between bracket fungus and flies, with particular emphasis on the relationship between *Fomes fomentarius* and *Myennis octopunctata*

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Keywords: bracket fungi, flies, spore dispersal

Bracket fungi disseminate their spores usually by wind, but this is not always effective – especially when the distance between host trees is significant, or host trees are spread in the complex plant communities. The role of invertebrates in dissemination is often underestimated. Arthropods play a role as spores vectors for some bracket fungi - most described in the literature are saproxylic beetles, less studied is the support of spore dispersal by flies, mites, and springtails.

The interesting relationship between *Fomes fomentarius* and *Myennis octopunctata* was observed by the team from the Botanic Garden of the University of Warsaw. It has been shown that the surface of the wings is a platform that makes the transfer of spores possible and even enables the dikaryotization of the mycelium (Faliszewska, 2008). The grooming, which is characteristic of the flies, enables the deposition of spores on uninfected branches and trees. It was observed that flies feed on the hymenophores especially intensely during the season of the spores liberation.

The meaning of the arthropod's activity is difficult to quantify accurately. The examples of experiments are presented on the poster.

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Fungal biodiversity data – where are they hidden?

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Keywords: fungal diversity, GBIF, data management

Although only 2-8% of all assumed fungal diversity is described, Fungi are considered one of the most speciose group of eukaryotes. They can be found in nearly all ecosystems and in the majority of terrestrial ones they play crucial roles. The knowledge of their diversity, distribution, and biology are crucial to facing current global challenges, such as e.g. climate change. Global Biodiversity Information Facility (GBIF, www.gbif.org) is an international network and data infrastructure funded by the world's governments and aimed at providing anyone, anywhere, open access to data about all types of life on Earth. However, occurrences of Fungi represent only ca. 1% of all reported occurrences. Therefore a global boost in fungal biodiversity data sharing is needed.

Based on the data collected in the Biological Diversity of Poland series (2003-2009) there are approximately 11,000 fungal species known from Poland but only 5292 are represented in GBIF by at least one occurrence [1]. Interestingly, 10% of these taxa were added via *iNaturalist* application [2], mostly by amateur mycologists. With the ongoing development of molecular techniques (including next-generation sequencing) and bioinformatics, the development of common standards and mechanisms for collecting data on the diversity of Fungi should become a priority.

Although 'FAIR Guiding Principles for scientific data management and stewardship' were published more than 5 years ago and the majority of European science funding agencies demand a description of data management systems, the majority of mycologists lack competencies in this field. Although the need to shape these skills within the curricula should be discussed, each of us can develop our skills by ourselves, e.g. by using:

<https://www.gbif.org/composition/2gdDUG4OI4rrduJtnz8j43/training-and-learning-resources>
or <https://plutof.ut.ee/#/manual> or <https://plutof.ut.ee/#/videos>

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Evolution of intraspecific diversity of *Phytophthora alni* subsp. *alni*

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Keywords: Peronosporomycota, phytopathology, *Phytophthora*, alder dieback

Phytophthora alni subsp. *alni* (*Phytophthora* × *alni*) is an invasive pathogen of alders (*Alnus* spp.), to this day known from Europe, which was initially described in 1995. It is a triploid and also the most significant taxon from the broader hybrid complex *Phytophthora alni sensu lato*. In present days, this invasive taxon is gradually spreading across Europe, causing significant environmental and economic losses. It spreads mainly via watercourses (zoospores) and with infested nursery stock. The population structure of this taxon in Europe is still evolving, and new genetic lineages have been recently recognized, however only one lineage makes up more than 50% of the overall population. In our work we would like to clarify the evolution of the genetic structure of *Phytophthora alni* subsp. *alni* populations during the invasion in the Czech Republic. We are using older *P. alni* isolates (collected around 2007 on the disease front, newly invaded area) and newly obtained isolates from the same area (present post epidemic phase) to compare their genetic structure. We are genotyping them using microsatellite analysis (collection of 10 microsatellites) and we are identifying multilocus genotypes. We will determine differences in population structure and hopefully we will also describe expected development of this taxon.



Fungal laccases in pharmaceutical industry and medicine

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Keywords: laccase, fungal enzymes, wood degrading fungi, biotechnological applications,
medicine

Laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) are glycoproteins containing four copper atoms, being distributed in all domains of life. The largest number of laccases is of fungal origin. These enzymes catalyse the oxidation of a wide variety of aromatic compounds coupled to the four-electron reduction of molecular oxygen to water. The use of oxygen (a non-limiting electron acceptor) by the laccases makes these enzymes adequate for industrial and environmental applications [1]. They are very useful biocatalysts for the pharmaceutical sector because they can catalyse a wide range of synthetic reactions, from the transformation of antibiotics to the derivatization of amino acids and the synthesis of metabolically stable amino acid analogs. Fungal laccases have been also studied in relation to the development of biosensors, which were developed in biomedicine for the detection of insulin, morphine, and codeine. Furthermore, it has been recently proved that these enzymes have antibacterial, antiviral, and anticancer potential [2, 3]. This work presents various medicinal roles of fungal laccases and gives perspectives for future studies of these biotechnological important enzymes.

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The occurrence of crown-tipped coral fungus *Artomyces pyxidatus* in Poland and assessment of its threatened status

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Keywords: fungi in ecosystems, *Artomyces pyxidatus*, deciduous wood

The basidiomycete *Artomyces pyxidatus* (*Russulales*, *Auriscalpiaceae*) is a lignicolous, coralloid fungus found throughout temperate regions of the Northern Hemisphere. It is included in the Red Lists of Fungi in Poland and some other countries. The current distribution of the species in Poland is presented, based on literature data as well as information submitted to the “Register of protected and endangered mushroom species” launched on the forum website <https://www.bio-forum.pl/messages/7259/7259.html>. In principle, all available published knowledge on the species has been compiled and analysed. The distribution of *A. pyxidatus* in Poland is mapped, and its regional ecology and relationships are discussed, including preferences of habitats and substrates, phenology, and threats. Based on the available information, it can be stated that the *A. pyxidatus* is closely connected to the rotten and decaying wood of logs and stumps, almost exclusively of deciduous trees. The compiled records confirm some ecological plasticity of the species, which can be found primarily in more or less natural broadleaved and mixed habitats (often protected in nature reserves), but also in managed – mixed, or even in coniferous forests. Based on the compiled distribution data for *A. pyxidatus* in Poland, a change of its Red List category is proposed.



The effect of herbicides on the infectious potential of entomopathogenic fungi

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Keywords: entomopathogenic fungi, herbicide, extracellular enzymes, 2,4-D, glyphosate

Entomopathogenic fungi are an important agent of the natural control of insect populations, therefore they can be used as biopesticides in fields and in greenhouse crops. Biological pest control, on the other hand, is not as popular as chemical pest control. The toxic chemicals commonly used are mostly difficult to degrade and penetrate deeply into the soil. Pesticide residues can adversely affect microorganisms present in contaminated soil.

One of the important steps in the process of insect infection by the fungus is the production of extracellular enzymes from the groups of proteases, chitinases, and lipases. It can be concluded that the infectious potential of entomopathogens is determined by the secretion of these enzymes. Therefore, these studies focused on the determination of extracellular enzyme activity in selected fungal strains. The popular herbicides 2,4-D and glyphosate in various concentrations were added to liquid cultures. The herbicides studied have not been shown to significantly affect the production of enzymes, which means that they do not reduce the infectious potential of entomopathogenic fungi. There was also no evidence of herbicide degradation by the fungal strains tested. This means that these herbicides and entomopathogenic fungi can be used simultaneously or at short intervals without significantly affecting their activity.



Community diversity and stress tolerance of culturable endophytic fungi associated with different tissues of *Phragmites australis* around Urmia Lake saline soils

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Keywords: Ascomycota, Biodiversity, Phylogeny, Salinity, Symbiosis

The endophytic fungi are considered to act as ameliorators of host fitness. In this study, we investigated biodiversity and salinity/pH tolerance of culturable endophytic fungi associated with different tissues of *Phragmites australis* around Urmia Lake saline soils. A total of 750 cultivable strains were isolated from 9600 plant tissues sampled from 16 sites around the Urmia Lake. Phylogenetic analyses based on partial 18S rDNA and the multi-locus analysis classified the recovered endophytic strains into 80 species within 11 orders belonging to Ascomycota and Basidiomycota. The order Hypocreales (Ascomycota) encompassing 375 strains belonging to 36 species was identified as the dominant order with highest frequency across the plant tissues and sampling sites. The Eurotiales (Ascomycota) took the second place with 125 isolates belonging to 16 species. The Eurotiales (Ascomycota) took the second place with 125 isolates belonging to 16 species. Endophytic fungal strains were subjected to growth response in pH gradients and salinity. Endophytes expressed different sensitivity to raised salinity (NaCl and KCl) and pH gradients. As a result, Eurotiales endophytes were the successful strains in tolerance against pH gradients and ionic conditions. However, some species from other orders also had good performance against salinity/pH gradients but this concert could not be generalized to the whole group. In the next step, the potential role of the obtained endophytic strains in promoting the growth of barley and rice under salinity stress are being investigated



Yeasts in the treatment of liquid digestate

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Keywords: yeasts, liquid digestate, treatment of digestion effluents

Anaerobic digestion is a technology in the green energy economy that enables the processing of organic waste generating two important by-products – biogas and digestate. The digestate can be separated into a liquid and a solid fraction. Whereas the treatment of the liquid digestate is much more difficult, the solid fraction can be processed into value-added products or heat.

Treatment of digestion effluents is a multifaceted problem resulting from the high content of carbon, nitrogen, and phosphorus compounds. The simplest method of the liquid digestate processing is its direct spreading over agricultural land. Due to its high costs and a negative impact on the environment, land application of the digestate is limited, and new treatment methods are being sought.

The use of yeast for the digestate treatment is a relatively new approach, and the research in this field is scarce. It was reported that some taxa, including *Saccharomyces cerevisiae* or *Yarrowia* sp., *Rhodotorula* sp., *Hansenula* sp., *Candida* sp., possess the ability to assimilate contaminants present in post-digestion effluents. They can effectively reduce high loadings of chemical oxygen demand and contribute to the reduction of the dark color of the effluent. After the treatment with yeasts, the liquid digestate can be reused for biogas production to dilute raw waste, lowering water consumption and wastewater streams generated in biogas plants. The proposed solution is strictly in line with the circular economy concept.



Efficacy of symbiotic modification of barley *Hordeum vulgare* and its impact on seedlings' growth

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Keywords: symbiotically modified organisms, crops, endophytes

Symbiotically modified organisms (SMO) might be a solution for arising problems regarding crop infections caused by fungal, bacterial and viral pathogens. The aim of this study was to (1) assess the efficacy of introduction (inoculation) of the fungal symbiont *Epichloë typhina* into barley *Hordeum vulgare* and (2) to verify symbiont's impact on plant size.

Two cultivars of barley were used in the experiment: Golden Promise and Roland.

Seeds lacking any endophytes originated from barley cultivation conducted in the Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań. Symbiont was introduced into 200 seedlings (100 of each cultivar). The procedure of inoculation consisted of making an incision in plants epidermis and insertion of viable fungal mycelium that was a *Epichloë typhina* laboratory strain. The control trial was also 100 seedlings of each cultivar. Those plants were cut in the same way, yet instead of introducing the mycelium they were injected with distilled water. Seedlings grew in a controlled environment inside a phytotron. After 42 days, the efficacy of inoculation was verified with two methods: (1) by staining with aniline blue prior to microscope analysis and (2) conducting the Petri dish culture method, in which leaves of the tested plants were cut and put on a medium. The Golden Promise cultivar yielded better inoculation efficiency. The symbiont's positive impact on the seedlings' growth was evident in both tested cultivars.



First report of *Sydowia polyspora* as endophyte of bryophytes

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Keywords: *Sydowia polyspora*, fungi, endophytes, bryophytes

Sydowia polyspora is one of the most common epiphytes, endophytes or pathogens of conifers worldwide [1]. *S. polyspora* is often associated with *Pinus* spp. and *Abies* spp. [2]. In our studies focusing on endophytes of bryophytes, *S. polyspora* was isolated from *Polytrichum commune* and *Polytrichastrum formosum*. The identification of this species is based on morphological traits and molecular data. This is the first time detection of *S. polyspora* as an endophyte of bryophytes. *Sydowia polyspora* is considered to be the primary colonizer of forest litter which enables its transmission into other organisms, not only conifers but also insects [3] and bryophytes as found in this study.

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Role of biosurfactants and volatile compounds produced by *Bacillus* strains in *Fusarium sambucinum* growth inhibition

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Keywords: *Bacillus*, *Fusarium*, antifungal activity, lipopeptides

Some *Bacillus* strains show antifungal ability due to production of cyclic lipopeptides classified as biosurfactants, as well as volatile compounds. The aim of the research was to compare the antifungal activity of *Bacillus* bacteria (grown in liquid and solid media) against *F. sambucinum* DSM 1094F. In the study, four *Bacillus* strains (Kol B2, Kol D3, Kol L2, Kol S8) and a filamentous fungus strain *F. sambucinum* DSM 1094F were used. Analyses were performed using microscopic techniques, LC-MS/MS and MALDI-TOF/TOF techniques. The liquid cultures were grown in LB medium, for 72 h with a constant shaking (120 rpm) at 28°C. The microorganisms were grown for 10 days on solid medium in compartmentalized Petri dishes with a bacterial culture on one side, and fungal on the other. The surfactin and iturin production in all liquid *Bacillus* cultures was decreased in the presence of *F. sambucinum*. However, the fungal growth was significantly (from 40% to 73%) inhibited. The results showed that two of the tested *Bacillus* strains (when grown on plates with a center partition) increase more than 5-fold their colony diameter in the presence of mycelium and inhibit aerial hyphae growth and spore production. The results indicate that all examined *Bacillus* strains, when grown in liquid media with *F. sambucinum* mycelium addition, keep their ability to produce biosurfactants and inhibit fungal growth. *Bacillus* strains Kol D3 and Kol S8 were determined as antifungal volatile compounds producers.



Fungal endophytes of the plant in a gradient of altitude: diversity and efficiency of vertical transfer

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Keywords: fungal microbiome, vertical transfer, endophytes

The composition of the fungal endophytes that colonize the plant is not constant and varies even between particular specimens of a species growing in the same area. The elevation gradient may be one of the factors that influence the diversity of the fungal endophytes in the plant. In these studies two hypotheses were tested: (1) the diversity of the fungal endophytes decreases with the increase of elevation and (2) the vertical transfer of the fungal endophytes from the mother plant to the seeds is not perfect. The material consisted of leaves and seeds of five plant species found in the Tatra National Park, from 1000 to 1750 m a.s.l.: *Soldanella carpatica*, *Homogyne alpina*, *Mutellina purpurea*, *Senecio subalpinus* and *Senecio nemorensis*. The obtained endophyte isolates were grown on plates containing PDA and chloramphenicol. Isolates representing the individual morphotypes were identified with the molecular markers ITS1 and ITS2. A total of 16 species of fungal endophytes were detected in five plant species. Five taxa were not present in the seeds, although they occurred in the leaves of the mother plants. The differences in altitude were not related to the endophyte composition observed on the test stand (Mantel test, $r=0.074$, $p=0.408$). In summary, the results show that plants have a similar composition of the fungal endophytes regardless of the altitude. The detected fungal species show no preference for altitude and the vertical transfer of the fungal endophytes is not perfect.



Application of the CRISPR/Cas9 system in genome editing of filamentous fungi

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Keywords: genomes editing, fungi, genetics

Filamentous fungi are an integral and important part of human life. They are applied in many areas of modern biotechnology including the agriculture and food industry, medicine, pharmacy, and many others. The constant development of science not only leads to the discovery of new fungal species but also to the new possibilities in terms of genetic manipulation of their genomes. However, traditional genetic methods are frequently time-consuming and accompanied with many factors that can contribute to the failure of an desired genome modification. The solution to these problems is the CRISPR/Cas9 system, which has revolutionized the biological and biotechnological sciences, and creates huge potential also for filamentous fungi. The aim of this work was to present the most important information on the operation of the CRISPR/Cas9 system in the context of fungal genomes editing, vectors construction and factors affecting the efficiency and accuracy of the system. Examples of application of CRISPR/Cas9 technology for fungal genome modification were demonstrated. Important aspects related to the constraints of this technology and the prospects for its future development and application were also discussed.

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