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INSTITUTE OF
AGROPHYSICS
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BOOK OF ABSTRACTS

Conference materials from 4th the conference

„MycoRiseUp! Youth in Mycology”

20-21 MAY 2023

Institute of Agrophysics Polish Academy
of Sciences in Lublin

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Michał Pylak

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KEYNOTE LECTURE

Bio(techno)logical strategies including plant holobiont and soil mycobiome for sustainable agriculture

Magdalena Frac

Institute of Agrophysics, Polish Academy of Sciences

m.frac@ipan.lublin.pl

Key words: Dysbiosis, Eubiosis, Microbial biodiversity, Plant holobiont, Soil mycobiome

Sustainable cropping strategies are relevant to maintaining the quality of the agricultural environment and are used to develop biotechnology solutions for sustainable and organic farming. Microbiomes play crucial functions for plant physiology and ecology (Dastogeer et al., 2020) as well as are essential for soil stability and resistance to biotic and abiotic stresses (Coleman-Derr and Tringe, 2014).

Microbial ecosystem balance of agroecosystems refers to soil-plant-microbiome interactions as critical step of eubiosis and dysbiosis states of plants and soil health maintaining (Berg et al., 2020; Frac et al., 2022). Moreover, fungal trophic modes and networks of mycobiome are important for plant and soil health (Siegieda et al., 2023). Healthy plants are associated with microbiomes by exchanging signal molecules, exudates, nutrients and other active compounds (Berg et al., 2017). Microbial-based solutions can be used as promising biotechnological strategy to reverse biodiversity loss and to restore soil health (Mącik et al., 2023). What is more biological conception based on biopreparations and the strategies including resource-use efficiency through microbiome interaction are needed to create healthy and more sustainable agroecosystems (Frac et al., 2018, Pylak et al., 2023).

Due to the complexity of the soil microbiome and plant holobiont, there is great uncertainty about how soil microbial communities are shaped by different crop species and agricultural systems. Therefore a new vision of agriculture which should include close connection with the microbiome of soil and plant and should put more attention to keep the microbial biodiversity of agroecosystems, still is challenging and needs research. Plant and soil microbiome management should be used and developed for increasing the resistance of certain plant crops to pests, pathogens, drought or nutrients.

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SESSION I : BIOTECHNOLOGY OF FUNGI

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Assessment of herbicides effect on *Bacillus* antifungal activity against a phytopathogenic *Fusarium sambucinum* strain

Aleksandra Walaszczyk¹, Weronika Rzeźnikowska²,

Anna Jasińska², Przemysław Bernat², Katarzyna Paraszek²

¹ Doctoral School of Exact and Natural Sciences, Department of Industrial Microbiology and Biotechnology, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland;

² Department of Industrial Microbiology and Biotechnology, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland

aleksandra.walaszczyk@edu.uni.lodz.pl

Key words: *Bacillus*, *Fusarium*, antifungal activity, herbicides, synergistic effect

Crop pathogens and pests reduce the yield and quality of agricultural production on a significant level every year. *Fusarium* is one of the top ten phytopathogenic fungi. These fungi have a high adaptive potential to infect various agricultural plants. Modern agricultural practices still rely heavily on synthetic pesticides to control plant diseases, however the application of these fungicides has raised concerns regarding human and environmental health. Biofungicides have been increasingly introduced as alternatives to the synthetic counterparts. Biosurfactants produced by *Bacillus* (surfactin, iturin and fengycin) have emerged as biological control agents on a sustainable agricultural basis. Some studies have indicated that bacteria from genus *Bacillus* can tolerate many chemicals, including pesticides, and even exhibit synergistic effects when applied in combination with them.

The aim of the research was to assess 2,4-D and metolachlor effect on *Bacillus* Kol B2 biosurfactant production and antifungal activity against a phytopathogenic *F. sambucinum* DSM1094F. Analyses included: assessment of fungal biomass growth inhibition, fungal lipid peroxidation and membranes permeabilization and *Bacillus* biosurfactants production.

The results showed, that contrary to both herbicides which promoted fungal growth, Kol B2 bacteria inhibited the fungal biomass by ~40%. Bacterial antifungal activity was enhanced by 2,4-D by 10%, and reduced by metolachlor. The Kol B2 production of biosurfactants was reduced in the presence of the mycelium. However, 2,4-D and metolachlor modified the observed inhibitory effect. In the presence of Kol B2 the fungal membranes permeabilization increased but no lipids oxidization occurred.

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This research was funded by the University of Lodz Doctoral School of Exact and Natural Sciences.

Can fungi grow on pens? Fungi on plastics

Dorota Wiktorowicz

Institute of Evolutionary Biology, Biological and Chemical Research Center, Faculty of Biology, University of Warsaw, Żwirki i Wigury 101, 02-089 Warsaw

d.wiktorowicz@student.uw.edu.pl

Key words: Plastics, biodegradation, *Paecilomyces variotii*

Every year humans produce over 300 million tons of plastics. Slow plastics degradation leads to the accumulation in the environment and we don't yet have any effective method of utilizing it. One of the promising technologies is microbial biodegradation, where microbial extracellular enzymes short polymer intermediates, so they can be used by microorganisms as a carbon source. We currently know more than 400 species of microorganisms that are capable of plastic degradation, including members of Ascomycota, Basidiomycota, and Mucoromycota.

When a pen covered with mycelia ended up in our laboratory, our first thought was that the fungus was growing on the ink. To identify the species, spores were placed on a PDA agar plate. As a result of an unfortunate accident, the Petri dish fell to the ground but did not break, so cultivation continued. After 4 days of incubation at room temperature, growth was observed not only on the agar but also on the lid of the plastic dish. ITS region amplification and sequencing have been performed and the fungus was identified as a *Paecilomyces variotii* sensu lato. *Paecilomyces variotii* Bainier is a commonly occurring species but causes opportunistic mycosis. We tested our strain for growth at 37°C, a temperature that is often used to check if microorganisms are potentially pathogenic. After observing growth we ended the experiment.

Pathogenic fungi cannot be widely used as plastic degraders because of the threat they pose. However, characterizing their metabolic pathways and enzyme production can contribute to the development of efficient plastic-degradation methods.

PUFA production in food fermentation by *Mucor* sp.

Grzegorz Ostrowski¹

¹ University of Warsaw, Faculty of Biology, Institute of Evolutionary Biology

g.ostrowski@uw.edu.pl

Key words: *Mucor*, PUFA, fermentation

With a growing human population, challenges of acquiring necessary nutrition from food are arising. Among various dietary elements are lipids including PolyUnsaturated Fatty Acids (PUFAs). PUFAs are not only necessary for proper brain development but also are an important source of antioxidants significantly lowering risks of cancers [1]. Commonly consumed sources of PUFAs are marine animals which are often harvested in an unsustainable manner [2]. The other are various plants constituting the majority of currently consumed oils. One disadvantage of plant based sources is that they may not have a proper balance of omega 3 PUFAs and sterols necessary for human dietary requirements [3]. Solution to that problem may be an initial fermentation of these plant based products by fungi which not only produce proper combination of fatty acids for human consumption but also enrich these plant based food in vitamins and macro nutrients naturally occurring only in meat [1]. Interesting group of the microorganisms producing PUFAs are fungi of the *Mucorales* order, especially the *Mucor* genus. Recent research provides data for both gamma-linolenic acid and alpha-linolenic acid production in psychrophilic *Mucor flavus* strains [1] which was also observed in the strain isolated in our laboratory. The representatives of this species are already used for food fermentation in the traditional Chinese cuisine [4].

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Endophyte eradication technology in the production of symbiotically modified grasses

Jean de Dieu Muhire¹, Dariusz Pańka², Jan Mućko²

¹ The Plant Breeding Company Grunwald Ltd., Group IHAR, Mielno 163, 14-107 Mielno, Poland

² Bydgoszcz University of Science and Technology, 7 Kaliskiego Av., 85-796 Bydgoszcz, Poland

mujados88@gmail.com

Key words: Symbiotically modified grasses, endophytes, *Epichloë*, cold atmospheric plasma, endophyte eradication

Microorganisms such as mycorrhizal, arbuscular fungi: *Rhizophagus* spp., *Glomus* spp. and endophytes of the genus *Epichloë* create symbiotic associations with grasses, which stimulate plant's higher resistance to numerous stress factors. The mutualistic symbiosis of the endophyte and the grass confers a number of benefits for the host plant such as pathogens, insects and nematodes resistance, drought tolerance, and improved competition with other plant species. However, grasses inhabited by wild endophytes can pose a threat to livestock due to the production of toxic compounds. "Safe" associations are those inhabited by selected endophytes, the so-called novel endophytes. Nevertheless, the introduction of such endophytes to the plant requires prior removal of toxic, "wild" endosymbionts naturally occurring in the cultivar. The purification process is challenging and time consuming activity. It is usually carried out with the use of fungicides or high temperature. Therefore, it is purposeful to search for new, more effective and environment friendly methods. So, research was started on the development of a new technology for eradication of those endophytes using cold atmospheric plasma (CAP). Currently, work is underway to optimize the operating parameters of the plasma generator, i.e. power and exposure time. The research is conducted on perennial ryegrass. Decontamination activity of CAP is analysed using microscopy method, laboratory tests on Petri dishes with PDA (Potato Dextrose Agar) medium and molecular methods. Treated seeds are also sowed into pots filled with peat substrate to determine the effectiveness of the method used for eradication of the endophyte.

Biosurfactant production of *Mucoromycota* representatives

Maksymilian Nowak, Julia Pawłowska

Institute of Evolutionary Biology, Biological and Chemical Research Centre, Faculty of Biology, University of Warsaw

me.nowak6@student.uw.edu.pl

Key words: Mucoromycotina, Mortierellomycotina, Bioremediation, Screening

Biosurfactants are amphiphilic compounds reducing interfacial tension between liquid phases. They have many advantages over their petrochemical counterparts. They are more chemically stable, less toxic and can be produced from waste material such as used frying oil from restaurants. Biosurfactants are used in many branches of the industry (textile, medicine, agriculture, chemistry) but also in soil bioremediation. They are produced by diverse microorganisms. Fungi produce a wide array of biomolecules, among them earlier mentioned biosurfactants. *Mucoromycota* is generally an understudied phylum within the fungi kingdom, but there has already been evidence that species belonging to this group can produce biosurfactants. In this study 69 strains of terrestrial *Mucoromycota* (67 from *Mucoromycotina* and 2 from *Mortierellomycotina*) were tested for production of such molecules using simple screening methods (Emulsification Index 24, Parafilm M Test and Oil Spreadin Test). Extensive production of biosurfactants by some of tested strains shows biotechnological potential of *Mucoromycota* that is yet to be discovered.

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SESSION II : BIOLOGY AND ECOLOGY OF FUNGI

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PLENARY LECTURE

Can entomopathogenic fungi decontaminate toxins?

Sylwia Różalska

University of Lodz, Department of Industrial Microbiology and Biotechnology

sylwia.rozalska@biol.uni.lodz.pl

Key words: Entomopathogenic fungi, biopesticides, biodegradation

Entomopathogenic fungi are a group of pathogens that infect and kill insects. They are important in natural pest control, as well as in the development of biopesticides. The most common genera of entomopathogenic fungi are *Metarhizium*, *Cordyceps*, and *Beauveria* (Litwin et al, 2020). These fungi infect insects by attaching to their exoskeletons and growing into their bodies, eventually causing death. As biopesticides, entomopathogenic fungi can have a significant impact on insect populations, and are used in agriculture and forestry to control pest insects, limiting the use of chemical insecticides. They are also being studied for their potential in biotechnology.

The soil environment in which entomopathogenic fungi inhabit is rich in various types of contaminants of both anthropogenic and microbiological origin. Entomopathogenic fungi come into contact with toxic substances found in the environment as a result of human activity (e.g. pesticides), but also with substances produced during intensive metabolic processes in microorganisms (e.g. mycotoxins). Therefore, knowledge of the biological mechanisms of the response of the fungi to these toxins is an extremely important issue. Entomopathogenic fungi have the ability to biotransform or even degrade a variety of toxic compounds in addition to their role in pests control (Nowak et al., 2019 and 2023). The possible use of these fungi for the bioremediation of contaminated sites is an active area of research, and holds great promise for environmentally friendly solutions to toxic waste management.

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Genomic characterization of *Umbelopsis* sp. and its endohyphal *Paraburkholderia* sp.

Alicja Okrasinska, Julia Pawłowska

*Institute of Evolutionary Biology, Biological and Chemical Research Center, Faculty of Biology,
University of Warsaw, Warsaw, Poland*

a.okrasinska@uw.edu.pl

Key words: bacterial-fungal interactions, endohyphal bacteria, *Mucoromycota*

Umbelopsis is a genus from one of the early diverged fungal phyla - *Mucoromycota*. Even though the exact ecology and trophic mode of its representatives remain elusive, these fungi are mainly considered saprotrophic soil microorganisms. In our previous research¹ we conducted a PCR-based screening that showed that the majority of *Umbelopsis* strains coexist with various bacteria, most notably endohyphal *Paraburkholderia* (*Burkholderiaceae*, *Proteobacteria*). Detected *Paraburkholderia* sp. are non-obligate symbionts of *Umbelopsis* as they are easily cultivable on agar and liquid media. Their role in the metabolism of *Umbelopsis* remains unknown, but endohyphal bacteria are generally known to broaden metabolic capabilities of their hosts. In order to verify this hypothesis as well as characterize the relationship between *Umbelopsis* and *Paraburkholderia* on molecular level, we sequenced genomes of both partners. We used Illumina NovaSeq for the fungal genome sequencing which resulted in a fragmented, yet complete, as estimated by BUSCO, assembly. The size of the fungal genome is estimated to be 22.5 Mb and contains 8786 putative genes. For the bacterial genome we used MinIon Nanopore and Illumina MiSeq reads. This approach resulted in a nearly chromosome-level assembly of a cumulative size of 9 Mb and 11689 coding sequences. We observed that the bacterial genome is not reduced as is common among other endohyphal bacteria and that it resembles other closely related free-living *Burkholderiaceae* which further confirms our hypothesis of facultative nature of this relationship.

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New *Mucor* species from Sudety Mountains, Poland

Beniamin Abramczyk, Dorota Wiktorowicz, Alicja Okraśńska, Julia Pawłowska

Institute of Evolutionary Biology, Biological and Chemical Research Center, Faculty of Biology, University of Warsaw, Żwirki i Wigury 101, 02-089 Warszawa

b.abramczyk2@student.uw.edu.pl

Key words: New species, Mucoromycotina, rhizoids, molecular phylogeny

Mucor genus, which contains mostly fast growing soil saprotrophs, was described by Linnaeus in 1753. Up until today over 600 *Mucor* species were described making it the most numerous genus among *Mucoromycotina*. During biodiversity study in soil contaminated with heavy metals and hydrocarbons (from Wieściszowice, Kolorowe Jezioro) unknown *Mucor* species was isolated. Phylogenetic analysis based on six molecular markers, physiological tests and morphological observations were performed. DNA sequences derived from the isolate cluster with ITS sequence of other *Mucor* sp. strain isolated from soil in Greenland. Together they form a sister clade to *Mucor microsporus*, however the genetic distance (based on ITS rDNA) surpasses 10%. Moreover it has morphological features that distinguish it from *Mucor microsporus*. The new *Mucor* species produces rhizoids in temperatures higher than 24°C. It also forms two layers of sporangiophores producing two types of sporangia each different in size comparing to *Mucor microsporus*. The isolated species has ability to grow in a temperature as low as 4°C and in oligotrophic conditions (Water Agar medium). It was also proved to interact with bacteria. Based on our observations and amplicon data derived from UNITE database it can be concluded that the new *Mucor* species is present in soil in Poland and Greenland. Considering its ability to form rhizoids in elevated temperatures name *Mucor thermorhizoides* is proposed. Together with *Mucor microsporus*, *Mucor thermorhizoides* represents a fairly understudied phylogenetic lineage of *Mucor* genus.

Unraveling the unknown: New proteins in motility apparatus of *Chytridiomycota*

Aleksander Kossakowski, Anna Muszewska
*e-mail: jakub.krzyzskowski@stud.umed.lodz.pl

Laboratory of Fungal Bioinformatics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences

akossakowski@ibb.waw.pl musze@ibb.waw.pl

Key words: EDF, DUFs, Chytridiomycota, motility

Early Diverging Fungi (EDF) are a diverse and ancestral set of fungal lineages (Voigt et al. 2021). Recent advances in genomics have revealed similarities between EDF and animals in cytoskeleton components, suggesting gene loss in Dikarya and conservation in metazoans, which is linked to multicellularity differences between fungi and non-fungal Opisthokonta (Kiss et al. 2019). Many fungal proteins possess protein domains classified as Domains of Unknown Function (DUFs; Mistry et al. 2021). This study focuses on DUFs, absent from Dikarya and occurring predominantly in Chytridiomycota, a group of EDF, which retained many of the ancestral features shared with Metazoa (Kiss et al. 2019). These DUFs expand the list of proteins associated with cytoskeleton and motility present in flagellated fungi. In our study we use a wide range of functional annotation tools and databases, of which most prominent are MPIToolkit, InterPro and CASTp, complemented by an extensive literature screening. Those applied methods allow us to infer functional annotation for several families of DUFs that may be important for motility apparatus in Chytridiomycota.

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Revision of the genus *Umbelopsis* based on multigene phylogenetic analysis and morphology.

Justyna Borowiecka, Theodore Gionnas

University of Warsaw, Faculty of Biology, Institute of Evolutionary Biology

jm.borowiecka2@student.uw.edu.pl
theodoregionnas@gmail.com

Key words: *Umbelopsis*, molecular phylogeny, morphological analysis

The genus name *Umbelopsis* was first used in the literature in 1966 to describe a type specimen of *Umbelopsis versiformis* [1]. Initially, many of the species, which now belong to the genus, were classified as *Mortierella* due to some similarities, e.g. strongly reduced columella[3]. However, based solely on morphological features, the authors didn't agree on the taxons' assignment to either *Mucoraceae* or *Mortierellaceae*. In the beginning of the 21st century, first attempts to determine phylogenetic relationships of *Umbelopsis* using molecular methods were made. The new family *Umbelopsidaceae* was proposed and *U. isabellina*, *U. ramanniana* clades were distinguished. However, till now they are known to be polyphyletic. Therefore, we aimed for a taxonomic revision of the genus, including the delimitation of potential new species within the *U. ramanianna* clade.

The molecular phylogenetic analysis was based on three markers ITS, LSU and SSU. Preliminary morphological data unveils differences between newly described *Umbelopsis* sp. strains, characterized by subtle intraspecific and intrastrain variations. The macro-, microscopical and physiological differences support the species delimitation indicated by molecular phylogenetic analysis.

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Culturable fungal communities inhabiting cryoconite holes in Svalbard

Justyna Borzęcka, Rafał Ogórek

University of Wrocław, Department of Mycology and Genetics, ul. Przybyszewskiego 63/77, 51-148
Wrocław, Poland

justyna.borzecka@uwr.edu.pl

Key words: Svalbard Archipelago, cryoconite holes, micromycetes

Cryoconite holes on glacier surfaces are variously shaped, organically rich and water filled depressions with microbial diversity and activity [4], and they are of biogeochemical, ecological, and biotechnological importance [3]. Yet, it is still little known about their fungal inhabitants [2].

Due to harsh conditions, fungi are isolated from those areas less frequently, and belong mainly to psychrophilic and psychrotolerant species. Studies show that genera such as *Rhodotorula* (e.g., *Rhodotorula psychrophilica*, *R. svalbardensis*, or *R. glacialis*), *Cryptococcus*, *Debaryomyces*, *Torulopsis*, and *Candida*; the Pezizales order, represented by the genera *Choiromyces* (specifically *Choiromyces meandriformis*), *Hydnотrya* (*Hydnотrya tulasnei*), or *Verpa* (*Verpa bohemica*); and Pezizales-related Dothideomycetes (e.g., *Alternaria* sp.) or *Thelebolus* are, among others, isolated from such areas. Additionally, other species obtained in previous research were composed of micromycetes – such as *Penicillium* spp., *Phialophora* sp., *Cladosporium* spp., *Circinella* sp., *Chytridiomycota*, or the phylum Ascomycota, and, finally, basidiomycetous yeast, such as *Mrakia* sp. (e.g., *M. robertii*), *Varicosporium elodeae*, or *Glaciozyma watsonii* [1].

The goal of this paper is to summarize the current research on culturable fungal communities from the Svalbard area, and to depict the importance of such studies.

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SESSION III : FUNGI IN INTERACTIONS & SESSION IV: PATHOGENIC FUNGI

Conference materials from 4th the conference

**„MycoRiseUp!
Youth in Mycology”**

20-21 MAY 2023

**Institute of Agrophysics Polish Academy of Sciences in
Lublin**

PLENARY LECTURE

Fungal biodiversity data - where are they? How to manage them?

Julia Pawłowska

*Institute of Evolutionary Biology, Faculty of Biology, University of Warsaw
ul. Żwirki i Wigury 101, 02-089 Warsaw, Poland*

julia.z.pawlowska@uw.edu.pl

Key words: biodiversity, GBIF, PlutoF data management system, FAIR data, open science

Ongoing climate change and increasing human impact on the environment made biodiversity monitoring one of the global priorities. Understanding trends and drivers of biodiversity shifts allows for identifying appropriate conservation measures but is impossible without easy access to the research-grade data on the species distribution. The Global Biodiversity Information Facility (GBIF), an international network and data infrastructure aims at providing open access to data about all types of life on Earth. Although fungi are the second most abundant group of eukaryotic organisms on Earth, playing a crucial role in the functioning of the majority of terrestrial ecosystems, the data on their distribution is scarce, fragmentary, and usually country-specific.

In Poland, data on the biodiversity of Fungi are traditionally published as national lists of occurrences. There are over 11,000 species, representing 122,333 occurrences from Poland published in GBIF now [1]. However, molecular techniques allow for monitoring the distribution of taxa, also the ones known only from DNA sequences, in a unified way across country borders. On the other hand, molecular data are lacking for many of the species described in the past. This leads to two datasets describing the same reality, yet with almost no intersection. Aware of this discrepancy, GBIF has just proposed a new data model [2] and the UNITE community [3] has introduced PlutoF data management system for biologists [4] to increase the efficiency of fungal biodiversity data sharing and integration.

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3. <https://unite.ut.ee/>
4. <https://plutof.ut.ee/#/>

Hidden in a fortress? - a distinctive *Mucoromycota* community of red wood ants' mounds

Igor Siedlecki^{1*}, Michał Kochanowski¹, Gabriela Reszotnik^{1,2}, Matylda Pawlicka¹, Marta Wrzosek¹

¹ Botanic Garden, Faculty of Biology, University of Warsaw, Aleje Ujazdowskie 4, 00-478 Warsaw, Poland

² Faculty of Agriculture and Ecology, Warsaw University of Life Sciences, Nowoursynowska 166, 02-787 Warsaw, Poland

*igor.siedlecki@uw.edu.pl

Key words: ant-fungal interactions, *Entomortierella*, *Formica polycтена*, microenvironments

Red wood ants are common insects of temperate forests, which live in self-build mounds. The mounds are composed mostly of organic matter (mainly coniferous needles) and are rich in antifungal substances (resin and formic acid). Such specific properties create a niche which could be beneficial only for some fungi present in temperate forests. Recent publications link *Entomortierella* and *Umbelopsis* with the red wood ant's environment^{1,2}. In our study we wanted to determine *Mucoromycota* taxa characteristic for mounds of *Formica polycтена* ants. In order to do that we collected samples two times, in pine forests around Warsaw. Samples were collected from the surface and inside of four *F. polycтена* mounds, together with surrounding forest litter. In our study, a culture-based method was employed with combined morphological and molecular (ITS and LSU) identification. Our results show that a *Mucoromycota* community of mounds differs from the one from the forest litter. *Entomortierella lignicola* and the '*Absidia* sct. *cylindrospora*' were found to be the indicator taxa for the mound environment, which suggests these taxa adaptation to the ant-made environment. *U. ramanniana* and *P. humilis* were associated with forest litter and rarely present in the mounds, which suggest that special properties of the ants' nests may work as a limiting factor for their occurrence. On the genus level, representatives of *Umbelopsis* were commonly isolated from both studied environments. Further studies, focused on the growth of the fungi in the mound-imitated environment could show and explain adaptations of mound-associated taxa.

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Wheat transcripts involved in plant-endogenous fungus *Chrysosporium pseudomerdarium* interactions

Polina Havrysh

Institute of Plant Genetics Polish Academy of Sciences, Department of Plant Microbiomics

havrysh.polina@gmail.com

Key words: endophytic fungi, wheat, gene transcription

Being one of the most grown agricultural crops, wheat is of the greatest importance to humanity as food source. (FAO, 2023). At the face of agroclimatic zones shift, it has become a major challenge to adopt local crops production to coming climatic changes. (Ceglar et al., 2019). One of approaches to accomplish this goal is using biological methods in crop protection, such as endogenous organisms. Endogenous fungi are known to benefit growth and fitness of various host-plants. (Waqas et al., 2012). In this study we tested how transcription levels change in wheat plants after interaction with endophytic fungi – *Chrysosporium pseudomerdarium* Oorschot.

For our experiment a common in Poland winter wheat (*Triticum aestivum* L.) variety – EUFORIA – was used, as well as strain of *C. pseudomerdarium* isolated from stems of wheat variety ARKADIA. (Salamon et al., 2023). Gene expression rates were measured for four genes of interest (GOI). To determine this parameter, we isolated RNA from 21-days axenic seedlings that were sprayed with a spores' suspension on 14th day, as well as from not sprayed ones for control. Isolated RNA was converted via reverse transcription to cDNA to then perform qPCR reaction with SYBR Green dye. Transcription levels of GOI were analyzed, comparing to ones of reference genes.

Results show that three studied GOI were up-regulated after fungi-plant interaction, compared to control, while the forth one was down-regulated.

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How to survive near a Ms. Clean? - *Entomortierella* adaptations to the ant-made environment

Siedlecki Igor*, Michalska Agata, Przygodzka Milla, Szeffler Katarzyna, Wierzbicka Maria,

Kochanowski Michał¹, Wrzosek Marta¹

University of Warsaw Botanic Garden, Aleje Ujazdowskie 4, 00-478 Warszawa

igor.siedlecki@uw.edu.pl

Key words: *Formica polycтена*, formic acid, pine resin, *Podila*, *Mortierellaceae*

Formica polycтена is an ant from temperate forests, which live in the self-build mound. Inside the mound, temperature is usually higher compared to outside. Mounds are composed of organic matter of nutritional value for fungi. However, ants, well known for their cleaning behaviors, spray it with formic acid and incorporate into it tree resin, to reduce the growth of microorganisms. Despite highly fungistatic properties of both substances, *Entomortierella* species are commonly isolated either from the ants of *F. polycтена* or their mounds.

In this study we wanted to check if ant-associated species: *E. lignicola* and *E. beljakovae* are adapted to live in habitat formed by ants in comparison to forest-litter associated *Podila humilis*. In order to verify it, we cultivated those species on substrates imitating the mound habitat and compared their speed of growth and intensity of sporulation to their development on a control medium.

Our results show that in the presence of formic acid: *P. humilis* is incapable of growing, growth as well as sporulation of *E. lignicola* are significantly stunned and development of *E. beljakovae* remains unaffected. Furthermore, while resin reduces the growth of all fungal species, higher temperature increases their rate of growth. What can be inferred from our data is that formic acid seems to be the most dominant factor shaping the mounds' fungal communities and *E. beljakovae* seem to be the best adapted to ant-made conditions. The scarce presence of *P. humilis* in the mounds can be explained by their low resistance to formic acid.

Fungal pathogens from invasive plants as a threat for native species

Paulina Pacek

Uniwersytet Przyrodniczy we Wrocławiu, Wydział Biologii I Hodowli Zwierząt

342126@uwro.edu.pl

Key words: fungal pathogens, native species, alien species

Invasive plant species can be a serious threat concerning occupying niches for native species, which is called a direct way of invasion. The indirect way is when invasive species act as a vector for pathogens. Such dynamics are called a spillover. A lot of attention has been brought to the effects of alien pathogens on human health or crop production. The effect on plants, animals, or even the whole ecosystem is usually put aside. It is believed that the alien pathogens disturb the native plant – native pathogen chain due to alien pathogens being generalists, meaning they can make more interactions per species, comparing to native pathogens, which are named as specialists. The question is: what do we know about such interactions and how dangerous they can be?

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Morphological changes of *Neosartorya* spp. (anamorph: *Aspergillus* spp.) observed under the influence of a food preservative

Wiktoria Maj, Giorgia Pertile, Kamil Skic, Magdalena Frac

Institute of Agrophysics, Polish Academy of Sciences

m.frac@ipan.lublin.pl

Key words: Filamentous fungi, Sodium metabisulfite, Scanning electron microscopy

Neosartorya genus includes several species, some of which are heat-resistant and have been found to produce mycotoxins that can contaminate food and feed (Yaguchi et al., 2012). In this study we investigated the effect of sodium metabisulfite, commonly used food preservative, on the morphology of 5 *Neosartorya* sp. isolates of varying sensitivity to natural extracts using scanning electron microscopy (SEM).

Neosartorya spp. were cultivated on Potato Dextrose Agar (PDA) supplemented with 150 µg/ml of sodium metabisulfite, and its morphology was compared with the control culture on PDA medium without the food preservative after 10 and 30 days of incubation at 30°C. SEM images showed differences in morphological reaction among the 5 isolates. We observed that the hyphae of tested fungi of *Neosartorya* genus grown in the presence of sodium metabisulfite were dotted with oval shaped structures and wider than hyphae of the control culture. Furthermore, isolates treated with the food preservative created more spores than fungi cultivated on the control medium. These morphological differences may suggest changes in the metabolic pathways or gene expression patterns of *Neosartorya* spp. induced by the presence of sodium metabisulfite.

Our results provide insight into the effects of sodium metabisulfite on the morphology of fungi and may contribute to the development of novel strategies against fungi belonging to *Neosartorya* genus in horticulture and agriculture, where sodium metabisulfite is rarely used.

The work was supported by the National Science Centre, Poland, Preludium Bis-2, 2020/39/O/NZ9/03421.

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SESSION V: Humans & Fungi

Conference materials from 4th the conference

„MycoRiseUp! Youth in Mycology”

20-21 MAY 2023

Institute of Agrophysics Polish Academy of Sciences in
Lublin

PLENARY LECTURE

Why and how to eat more mushrooms

Wojciech Radzki

*Sub-department of Fruits, Vegetables and Mushrooms Technology
Department of Plant Food Technology and Gastronomy
Faculty of Food Science and Biotechnology
University of Life Sciences in Lublin*

wojciech.radzki@up.lublin.pl

Key words: mushrooms, consumption, nutritional value, bioactive compounds, enriched food

On average, Polish people consume several kilograms of mushrooms per year. Both mushrooms collected from natural sites and those from commercial crops are eagerly consumed. The incorporating mushrooms into the daily diet is beneficial for humans mainly due to the high content of B vitamins, minerals and protein. In addition, mushrooms are a source of many bioactive ingredients with health-promoting effects: immunostimulating, anticancer, beneficial to the cardiovascular system, anti-inflammatory, antibacterial or antiviral. Epidemiological studies conducted on a large population of people indicate a beneficial effect of mushroom consumption on human health in the context of cancer or cardiovascular diseases. Due to the high nutritional value of mushrooms and unique sensory characteristics, manufacturers are increasingly using this raw material to produce fortified food. Mushroom fruiting bodies are used in the production of plant meat analogues, cereal products (pasta, bread, biscuits) or dairy products. Research is also being conducted on the possibility of using health-promoting ingredients isolated from mushrooms (e.g. β -glucans) as food additives. For the production of mushroom-enriched food, cultivated species seem to be a good raw material as their production is relatively cheap and fruiting bodies are available for the whole year.

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Medicinal properties of selected fungi occurring in the natural environment

Anita Veseli, Bachelor of Science in Medical Biology

Maria Curie- Skłodowska University, Department of Botany, Mycology and Ecology

anita.veseli011@gmail.com

Keywords: Fungi, medicinal properties, environmental health

Fungi have been utilized for their potential health benefits in medicine for thousands of years. Nowadays, we have significant research on the use of fungi in various areas of medicine. Some ways fungi are being investigated include their potential anti-cancer, anti-inflammatory, and immunomodulatory properties. They are also being explored for their use in therapies for conditions like depression, anxiety, and addiction. As our understanding of the benefits of fungi grows, they are becoming increasingly integrated into modern medicine, paving the way for new and exciting treatment options.

Gaining knowledge about the importance of fungi and their role in natural environments can enable their utilization of medicine in a manner that supports and strengthens medical treatments.

In my upcoming talk, I will be focusing on the medicinal properties of four different species of fungi found in the Basidiomycota phylum, as well as one species from the Ascomycota phylum. I will discuss why these particular species were chosen for my bachelor's thesis, and provide an overview of the main arguments presented in my research. I will also highlight the ecological significance of these fungi and their role in our environment. My goal is to inspire a greater appreciation for the value of fungi and their potential to improve both human and environmental health.

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Medicinal mushrooms as an alternative therapy for depression: A mini review

Jan Lazur, Kamil Hnatyk, Katarzyna Kała, Bożena Muszyńska

*Jagiellonian University Medical College, Faculty of Pharmacy, Department of Pharmaceutical Botany,
Medyczna 9 Street, 30-688 Kraków, Poland*

janlazur@gmail.com

Key words: depression, edible mushrooms, secondary metabolites

According to World Health Organization report published in 2017, more than 322 million people worldwide suffer from Major Depression Disorder (MDD) [1]. The pathophysiology of depression is complex and not fully understood – currently, pharmacotherapy of depression is based on monoamine theory of depression which states that MDD is caused by insufficient levels of serotonin, noradrenaline and/or dopamine [2]. Medicines used in pharmacotherapy of depression may reduce the reuptake of abovementioned neurotransmitters (like selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs)) or inhibit the activity of monoamine oxidase which break neurotransmitters down into non biologically active molecules. Unfortunately, despite the current developments in psychopharmacology, almost 30% of patients who suffer from depression are treatment resistant. Therefore, seeking for new intervention in prophylaxis, adjuvant therapy or novel compounds with antidepressant activity is a priority.

Edible mushrooms are a source of substances that exhibit multidirectional, beneficial to humans, biological activity. Recent studies suggest that systematic ingestion of edible mushrooms may lower odds of depression. However, the mechanism of antidepressant activity remains unclear [3].

The aim of the review is to suggest possible explanations of antidepressant activity of edible mushrooms. The following directions will be discussed: edible mushrooms as a source of serotonin precursors – L-tryptophan and its derivatives, edible mushrooms as a source of antioxidants and link with inflammatory based depression theory. In the end, the connection between gut microbiota, depression symptoms presence and edible mushrooms ingestion will be evaluated.

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The impact of storage on antioxidant properties and phenolic compounds content in *Boletus edulis* and *Imleria badia* fruiting bodies

Małgorzata Namiota, Radosław Bonikowski, Kamil Szymczak

Institute of Natural Products and Cosmetics, Faculty of Biotechnology and Food Sciences, Lodz University of Technology

malgorzata.namiota@dokt.p.lodz.pl

Key words: antioxidant ability, fungi decay, shelf-life, postharvest, mushrooms storage

Boletus edulis and *Imleria badia* are Poland's most commonly picked and consumed mushrooms. Their popularity stems from both their unique taste and their durability against quick deterioration. Usually, the fruiting bodies of these mushrooms can be kept for up to 2-3 days before they must be consumed or processed. However, we might observe quick changes in their appearance and texture due to the shelf-life – especially browning and firmness losses. Those changes are happening due to water loss, and microbial attacks, but especially – oxidative stress. The increase in the activity of polyphenol oxidase, which is responsible for enzymatic browning may cause phenolic compounds losses, resulting in a quick decrease in the antioxidant properties of mushrooms. We investigated the qualitative and quantitative composition of phenolic compounds in the fruiting bodies of these two species of fungi. The samples were dried immediately after picking and after 4 days of storage at room temperature using the GCxGC-TOFMS apparatus. Then, the extracts of fruiting bodies were subjected to the total phenolics analysis (Folin-Ciocalteu method) and antioxidant ability by DPPH* assay.

We found 9 phenolic acids and 1 phenolic aldehyde determined in extracts of *Imleria badia* dried fruiting bodies, while in *Boletus edulis* – 7 phenolic acids. Trans-cinnamic and phenylacetic acids accounted for about 90% of the total amount of phenolic compounds in *Imleria badia* and about 70% in *Boletus edulis*. We observed severe changes in the phenolic compounds content as well as the antioxidant ability of mushrooms due to 4 days of storage.

The effect of propolis on the formation of biofilm by *Candida* spp

Jakub Krzyszkowski^{1*}, Sebastian Stuczyński¹, Katarzyna Skrzypczak¹, Ksenia Urbaniak¹, Jarema Wódka¹,
Sandra Galant², Katarzyna Góralska², Ewa Brzezińska-Lasota³

*e-mail: jakub.krzyszkowski@stud.umed.lodz.pl

¹ Student's Scientific Society of Biology and Medical Parasitology, Medical University of Lodz

² Department of Biology and Parasitology, Medical University of Lodz

³ Department of Biomedicine and Genetics, Medical University of Lodz

Key words: *Candida*, biofilm, yeast, propolis

Fungi of the genus *Candida* are often found in human organism as a part of natural microbiota or pathogens, that cause candidiasis in states of weakened immune system. The formation of a biofilm belongs to important fungal virulence factors, which impairs the human autoimmune response and increases their resistance to antimycotics. Therefore, many studies are currently focused on methods of effective prevention and control of biofilm. The aim of the study was *in vitro* evaluation of potential inhibition of *Candida* biofilm formation by propolis solutions.

During the research, 8 reference strains (*C. albicans* ATCC 10231, *C. albicans* CB 2312, *C. albicans* L-45, *C. parapsilosis* ATCC 22019, *C. parapsilosis* CBS 16947, *M. guilliermondii* ATCC 6260, *C. krusei* CBS 573, *C. tropicalis* CBS 2424) and 3 alcoholic solutions of propolis from Polish apiaries (2 samples of propolis from Podlasie and 1 sample from Kaszuby) were used for the test. The biofilm formation inhibition was assessed using the tetrazolium salt reduction (MTT) test. Then, the most potent propolis was selected and a microscopic analysis of changes in the *Candida* biofilm structure in the presence of propolis was performed.

The *in vitro* tests showed the antifungal potential of alcohol solutions of propolis. Inhibition of biofilm formation was noticed in the concentration range (1.56-3.13 mg/ml, average 2.52 mg/ml). The strongest biofilm-inhibiting effect was demonstrated for propolis from Kaszuby. This propolis was used for microscopic analyses. Detailed results will be presented during the conference.

Fanconi Anemia pathway in Early Diverging Fungi

Drishtee Barua, Anna Muszewska

Laboratory of Fungal Bioinformatics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences
Pawińskiego 5a, 02-106, Warsaw, Poland

drishteebarua@gmail.com

Key words: Fanconi anemia, Early Diverging Fungi, Dikarya, in-silico, Transcriptomics

Fanconi anemia (FA) is a DNA repair pathway that recognises damage caused by DNA interstrand crosslinks (ICL). ICLs are one of the lethal forms of DNA damage that prevents separation of two strands of duplex DNA and blocks transcription and replication. A typical FA pathway consists of an activation complex, a core complex, an endonuclease and a repair complex working continually [1]. This pathway was initially thought to be restricted to vertebrates, but was later confirmed in metazoans. The identification of homologs of FANCM, FANCL and FANCI in yeasts opened the possibility of a rudimentary form of FA DNA repair system lacking a majority of FA core proteins [2]. In this work, we attempt to expand the search for FA components in the fungal kingdom using an in-silico approach. We identified homologs of four core proteins, seven endonucleases and four FA activation and repair proteins respectively. We noted the lack of several key components in all fungal taxa including FANCC, FANCF and FANCG responsible for post-replication repair and chromosome stability. The observed distribution can be attributed to a gradual loss of FA components from EDFs to Dikarya. While FANCD2 and FANCI responsible for the formation of the ID complex [3] required for a functional FA pathway are conserved in EDFs, they are lost in Dikarya. Using gene expression profiling of transcriptomic datasets, we also found 13 FA proteins overexpressed during stress. Our results might be an indicator of a minimal FA pathway in the EDFs, with gradual loss of their components towards Dikarya.

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POSTER SESSION

Conference materials from 4th the conference

„MycoRiseUp! Youth in Mycology”

20-21 MAY 2023

Institute of Agrophysics Polish Academy of Sciences in
Lublin

PLENARY LECTURE

Psilocybin and LSD – past and future

Marta Wrzosek

University of Warsaw, Botanic Garden

ma.wrzosek@uw.edu.pl

Key words: psychedelics, therapies, visual art, creativity

The use of psychedelic drugs throughout history for both ritual and medical purposes is well-documented across all continents. The popularity of these substances skyrocketed after the discovery of LSD by Albert Hofmann in 1938, followed by the description of psilocybin and psilocin in 1959, which greatly influenced the Hippie culture (Hofmann 1959, 1980). In 1971, the US government deemed psychedelics illegal as part of their "War on Drugs" hindering research on their therapeutic properties for many years. However, recent grants have been awarded to laboratories in the US and Europe to explore their medicinal potential. Research conducted by R. Carhart-Harris and D. Nutt and their teams have shown that psilocybin and LSD, when used in conjunction with psychotherapy (psychedelic-assisted therapy), can effectively treat certain mental disorders (Carhart_Harris et al.,2021, Nutt et al, 2021). Both substances are tested for therapeutic values in treating depression, PTSD, alcoholism, or cluster headaches. The results seem to be promising (Carhart-Harris and Goodwin, 2017) On the other hand, many people believe that psychedelics can enhance creativity, but scientific studies have not fully supported this claim, highlighting the importance of "set and setting" during a psychedelic experience.

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Production of harzianic acid by selected *Trichoderma* sp. species in the presence of metolachlor

Dominika Antosiak, Anna Jasińska

Bioethnology and Microbiology Students' Society "Bio-Mik", Faculty of Biology and Environmental Protection, University of Lodz, 12/16 Banacha St., 90-237, Lodz.

Department of Industrial Microbiology and Biotechnology, Faculty of Biology and Environmental Protection, University of Lodz, 12/16 Banacha Street, 90-237, Lodz.

dominika.antosiak@edu.uni.lodz.pl

Key words: *Trichoderma*, enzymes, fungi

Trichoderma is a genus of fungi that is known for producing enzymes and metabolites with potential applications in industry, agriculture, and the environment. They can inhibit the activity of phytopathogens attacking crop plants. This antagonism may result from competition for space and nutrient substrates, enzymatic lysis of pathogen cells (by enzymes such as chitinases, glucanases, and proteases) and production of antifungal secondary metabolites (including harzianic acid, T22-azaphilone, peptaibols). The antagonistic activity of *Trichoderma* fungi against plant pathogens may be disrupted by pollutants such as heavy metals, pesticides, and microplastics resulting from human activities. The present study assessed the production of harzianic acid by *T. harzianum*, *T. koningii* and *T. reesei* in the presence of metolachlor (MET), a commonly used chloroacetanilide pesticide. Among the tested fungi, only *T. harzianum* was able to synthesize this metabolite. Its content in the cultures containing 10 and 25 mg/l MET increased by about 10-20% in relation to the control system without herbicide. Harzianic acid production was maintained even at a concentration of 100 mg/l MET. These results may suggest that the tested microorganism retains the ability to synthesize antifungal secondary metabolites even in the presence of pollutants.

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The risk of infections of *Quercus* spp. by *Phytophthora* spp. in city parks

Joanna Barton, Jadwiga Tomalak, Zuzanna Polak, Magdalena Ogonowska, Pola Wasilewska

SKN Medyków Roślin “*Armillaria*”, Faculty of Life Sciences and Technology, Wrocław University of Environmental and Life Sciences

Faculty of Life Sciences and Technology, Wrocław University of Environmental and Life Sciences

124140@student.upwr.edu.pl

Key words: oomycota, *Quercus* spp., *Phytophthora*, phytophthorosis

Organisms of the genus *Phytophthora* are dangerous plant pathogens. They belong to the kingdom *Chromista*, of the phylum *Oomycota*, and are the culprits of plant diseases worldwide. They cause diseases called phytophthorosis on cultivated and ornamental plants as well as on trees, thus they pose a threat to farms, orchards, plantations and forest crops. In addition, many new species belonging to this genus have been described in recent years. Many of them cause mass dieback of trees in forests, for example *Phytophthora ramorum* is considered to be the spread of mass dieback of oak trees in North America, and which is already affecting many European countries. *P. ramorum* has been also identified in Poland, but there are also 11 other species of *Phytophthora* that parasitize on oaks have been identified in our country.

The aim of the study was to take a closer look at pathogens of this genus in terms of their ability to infect oaks (*Quercus* spp.) in urban plantings, e.g. in parks. Urbanization makes plants grow under conditions of shortage and stress, which weaken them and make them more susceptible to infection. In parks, due to the presence of native and alien species growing together, the phenomenon of phytophthora spillback may occur

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Fungal diversity of red wood ants (*Formica s. str.*) infrabuccal pellets

Izabela Bak*¹, Igor Siedlecki¹, Marta Wrzosek¹

¹ Botanic Garden, University of Warsaw, Al. Ujazdowskie 4, 00–478 Warsaw, Poland

ia.bak@student.uw.edu.pl

Key words: ant-fungal interactions, insects, infrabuccal pockets, *Formica polyctena*, microenvironments

The infrabuccal pocket is an organ located inside ants' head. Its main function is to prevent particles from entering further parts of digestive tract. The particles are collected and form a pellet while ants are feeding and grooming. The diversity of organisms present in infrabuccal pockets is still not completely studied. Earlier studies showed that infrabuccal pockets contain fungi and that in some ants species queens transport fungi^{1,2}. The aim of our work was to study the fungal diversity of infrabuccal pockets of the red wood ants, common insects of temperate forests. We studied infrabuccal pellets of 30 *F. polyctena* workers and 14 *Formica s. str.* young queens. Contents of the pellets were subjected to microscopic analysis with photographic documentation and morphological identification was carried out. Fungal material was found in all 44 pellets. Dark-filament fungi were found in 92,5% of the pellets, and the light-filament fungi were found in 86,5%. Fungal diversity in the pockets differed between individuals. In workers, the number of identified taxa varied from 4 to 16 per pellet. The most common morphological types were *Cladosporium*-like and *Penicillium*-like spores. While results of our study shows a wide variety of fungal forms present in the ants' infrabuccal pockets, it is still unknown what is the function of these fungi. This stimulates the need for further exploration. We plan to culture fungi isolated from the pockets, and to study the fungal diversity of larvae and pupae of ants.

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Stable Isotope Probing as an underutilized technique for tracking nutrient cycles in complex microbial communities

Mikołaj Charchuta

*Adam Mickiewicz University in Poznań, Department of Plant Physiology
Department of Botany, Faculty of Science, Charles University
Laboratory of Environmental Microbiology, Institute of Microbiology, Czech Academy of Sciences*

makchm@st.amu.edu.pl

Key words: carbon cycling, molecular ecology, meta-metabolome

The great plate count anomaly remains an insurmountable problem in functional studies of microbial communities. While new methods for its resolution are emerging, they have to be, by their very nature, limited in their scope. Metagenomics provides the broadest perspective and yet, it can still be improved upon with isotopic methods. The only limit to the latter being poor accessibility of radioactive reagents. Stable Isotope Probing, a technique based on supplementing the community with substrate enriched with a stable isotope. This change does not change nutrient uptake, but it enables its tracking through nucleic acids, which differ in their buoyant densities in organisms which utilize the studied substrate. The difference is what enables us to separate taxonomic groups, and functional genes/transcripts along the axis of substrate utilisation. In my poster I would like to present experiences with this technique, as well as highlight its future potential in microbiome research.

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Metabarcoding of *Quercus robur* mycobiomes from the 'Meteorite Morasko' Nature Reserve as a source of referential data for *Carpinion betuli* forests

Maksymilian Chmielewski, Mikołaj Charchuta, prof. UAM dr hab. Władysław Polcyn

Adam Mickiewicz University in Poznań, Department of Plant Physiology

makchm@st.amu.edu.pl

Key words: computational statistics, molecular ecology, profiling

So far macro- and microscopic surveys of fungi have been considered the most certain methods of assessing biodiversity. Nonetheless DNA-based taxonomy is steadily becoming more important & established as the state-of-the-art approach. Here we propose a simplified procedure based on bioinformatical methods validated by computational statistics to be used after high-throughput amplicon sequencing. Our procedure provides a general overview of taxonomic and quantitative data of soil microbiomes as well as deep insight into details such as what are key species, how all species fit into the network and whether the results are statistically relevant. It accomplishes that with the use of methods such as: differential network analysis, various clustering approaches, computational statistics and interactive taxonomic and functional composition visualization. This procedure enables useful biodiversity profiling of protected and disturbed areas. We tested the effectiveness of our method analysing mycobiomes of common oaks from 'Meteorite Morasko' Nature Reserve. We chose that reserve assuming it as an untouched soil ecosystem so that we can propose it as referential data for oak-hornbeam forests. After performing our method and computational statistical validation we found 820 relevant fungal species split into six plant-oriented ecological groups. We also constructed networks showing cooperation and competition interactions and biodiversity charts. Based on the results of that analysis we confirm that chosen reserve is suitable as a reference data set.

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Methods of fungal transformation

Agnieszka Frac, Łukasz Gregorczyk

Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology

agnieszka.frac@student.uj.edu.pl
lukasz7.gregorczyk@student.uj.edu.pl

Key words: transformation, fungi, methods

There are many known methods of fungal genetic transformation. At first methods for transformation of unicellular fungi were established to soon be followed by methods regarding multicellular organisms. Apart from traditional method using PEG/CaCl₂-mediated protoplast transformation, there are other more modern techniques such as: electroporation, biolistic method, and a variety of mediated by external DNA carriers methods. All of them have advantages and disadvantages, but one of the most commonly mentioned hindrances are difficulties in standardization said methods and the presence of cell wall. The fungi are transformed for two most important reasons first and foremost to produce useful metabolites for instance transformed *Aspergillus niger* is producing citric acid more efficiently than the wild type. The second application is mitigation of fungal pathogenicity.

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Effect of endohyphal bacteria on the growth rate of *Umbelopsis* sp.

Maria Furman¹, Mikołaj Dziurzyński², Alicja Okraśńska¹, Julia Pawłowska¹

¹ Institute of Evolutionary Biology, Biological and Chemical Research Center, Faculty of Biology,
University of Warsaw, Warsaw, Poland

² Department of Biology, University of Florence, Italy

m.furman8@student.uw.edu.pl

Key words: *Paraburkholderia* sp., Mucoromycota, symbiosis

Fungi and bacteria are found living together in all types of environments. Their interactions play a crucial role in shaping terrestrial ecosystems. Among all the interactions they engage in, symbiosis between fungi and their endohyphal bacteria is particularly interesting. Although there are many articles describing this kind of interaction, we still know very little about how endohyphal bacteria affect the growth rate of the fungal host in poor medium conditions. In this study we examined how endohyphal bacteria, *Paraburkholderia* sp., affects the growth rate of its host fungus, *Umbelopsis* sp. In order to do so, colonies of the same strain of *Umbelopsis* sp. harboring endohyphal bacteria (EHB+), and those treated with antibiotics (EHB-) were used for a comparative growth experiment. Liquid cultures were established from both types of colonies and for the next two weeks the medium was removed from one flask each day and the material was rinsed with distilled water. Samples prepared in this way were dried in 105 °C for 24 hours and then weighed. The dry-mass of the individual samples were measured and growth graphs were calculated. Preliminary results suggest that endohyphal bacteria, *Paraburkholderia* sp., have a positive effect on the growth rate of *Umbelopsis* sp. in conditions of mineral medium with glucose as the only carbon source.

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It's not limited to *Archaea* – on methane emissions in the world of fungi

Tomasz Grzyb

Department of Biology and Environmental Protection, University of Lodz

tomasz.grzyb@edu.uni.lodz.pl

Keywords: methane emissions, methane paradox, climate change, fungi, carbon cycle

Methane (CH₄) is a potent greenhouse gas, second only to carbon dioxide in driving climate change during the industrial era. CH₄ warming potential is 28 times more powerful than CO₂ in a 100-year timescale. Moreover, methane contributes to the formation of ground-level ozone, a dangerous air pollutant. The atmospheric amount of methane has more than doubled since pre-industrial times. To control and minimise the threat connected to methane emissions, we need to understand global CH₄ sources and the processes behind them.

Biogenic methane sources typically refer to archaeal methanogenesis, an anaerobic process playing a quantitatively important role in global methane emissions. However, the methane paradox is observed – although methanogenesis is expected to happen only under strictly anoxic conditions, peaks in CH₄ concentrations in oxygen-saturated waters are present. Increasing evidence has confirmed that cyanobacteria, algae, and fungi could produce methane in oxic environments.

Fungi not only enhance archaeal methanogenesis by initiating the degradation of organic matter, but they can also produce methane themselves. It has been shown that methane could be produced continuously under oxic conditions without any archaeal presence, i.e., by *Pleurotus sapidus* and *Laetiporus sulphureus*. It is possible to happen when various metabolites (such as methionine) are degraded, possibly as a stress response. The biochemical and physiological aspects of these processes, as well as the fungal role in the global methane cycle, remain mostly unclear. However, non-methanogenesis processes could be important contributors to global methane emissions. Here, we present current state-of-the-art concerning methane emissions in the world of fungi.

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Psilocybin revolution in psychiatry

Hanna Kaczmarczyk,

Mycologic Section of Student Naturalist Association of the Jagiellonian University
Jagiellonian University, Faculty of Biology

hania.kaczmarczyk@student.uj.edu.pl

Key words: psilocybin, mushroom, psychiatry, depression, treatment

Psilocybin is an alkaloid found in over 100 species of mushrooms. This substance is structurally similar to serotonin and is classified as a tryptamine. Its most significant quality is that it acts as an agonist of 5-HT_{2A} serotonin receptors, which makes it a potential drug for use in psychiatric treatment. These receptors play a role in regulating mood, cognition, and perception, and are also involved in the development of various psychiatric disorders. In the future, psilocybin may be used for depression, anxiety and addiction therapy.

One of the reasons psilocybin has shown promise as a treatment option is because it works differently than traditional medications. Instead of simply masking symptoms, it appears to address underlying psychological issues by promoting changes in perception and behavior. Specifically, it has been shown to increase emotional openness, decrease depressive symptoms, and reduce anxiety in clinical trials. Additionally, this substance does not seem to cause any long-lasting side effects and is not classified as addictive.

Still, there are many unknowns so the research in this topic is truly crucial. Further experiments are needed to fully understand the drug's therapeutic potential and to develop safe and effective treatment protocols as well as understanding of its long-term effects on the brain.

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Genome editing of *Saccharomyces cerevisiae* using CRISPR-CAS9 system

Anna Lisowska

Biotechnological and Microbiological Students' Scientific Association "Bio-Mik", University of Lodz,
University of Lodz

anialisowska567@gmail.com

Key words: *Saccharomyces cerevisiae*, CRISPR-CAS9, genome editing,

The yeast *Saccharomyces cerevisiae* is one of the basic model eukaryotic organisms in genetics and molecular biology. They are unicellular heterothallic microorganisms, belonging to the phylum of *Ascomycota*. Because of their simplicity, ease of culturing, and ability to carry out successful genome editing, eukaryotic organisms are a good model for studying metabolic pathways and gene activities. The *Saccharomyces cerevisiae* genome was fully sequenced in 1996 as the first eukaryotic genome.

Genome editing has become an important tool in biological research in recent years. It allows the precise modification of DNA sequences in living organisms, which enables a better understanding of the function of individual genes and their effects on various biological processes.

CRISPR-Cas (clustered regularly interspaced short palindromic repeats-CRISPR-associated protein 9) is a natural prokaryotic defense system that uses RNA to recognize and destroy genetic material of phages or other foreign DNA. Class 2 CRISPR/Cas9 from the bacterium *Streptococcus pyogenes* is used in genetic engineering. It consists of a Cas9 protein and a synthetic sgRNA that is a chimera of crRNA and tracrRNA, which enables precise cutting of DNA at target sites. With sgRNA, it has become possible to remove and insert any gene at selected locations in the genome. This method is used to remove, modify and add DNA sequences in the yeast genome. Since its discovery, CRISPR-Cas9 has quickly become the preferred genome editing technology for engineering the genome of yeast, as well as other organisms, due to its efficiency and versatility.

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Orchid mycorrhizal fungi (OMF) – Why are they so special and how can we use them

Jacek Łuc

*Mycologic section of student naturalist association of the Jagiellonian University,
Jagiellonian University, Faculty of Biology*

jacek.luc@student.uj.edu.pl

Key words: orchid mycorrhizal fungi, orchidaceae, germination, biological control

Orchidaceae family is one of the largest plant groups and has both an esthetical and economical meaning. Their seeds are non endospermous - that's why they are mycoheterotrophic, which means that in order to develop they get into symbiosis with orchid mycorrhizal fungi (OMF). This kind of relationship is very special among plants in general mostly because orchids have the ability to interact with many different fungi, even those who are pathogenic or parasitic. As many researchers show OMF not only interact with the seeds and protocorms but also with adult orchids. They affect the whole ecosystems, can provide key nutrients providing superior growth but also what is important for agriculture - they can protect plants from other pathogens causing dangerous decimating diseases. It's important for orchidaceae that are used for economic reasons like *Vanilla planifolia* or *Gastrodia* and others important for medicine. Extracts from some OMF are used for fighting with blights like rice blast disease. They are worth studying and can improve efficiency in many sectors of life.

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Could a beneficial fungi control plant pathogens? interactions between *Trichoderma* and *Verticillium*

Mateusz Maćk, Jacek Panek, Agata Gryta, Karolina Oszust, Michał Pylak, Magdalena Frąć

Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland

e-mail: m.frac@ipan.lublin.pl

Key words: plant pathogen, beneficial fungi, Nanolive's holotomography, mycoparasitism

Verticillium spp. includes a number of soil-borne phytopathogens affecting many species of fruits, vegetables, flowers and trees (1). Fungi belonging to the *Trichoderma* genus are considered as an effective biocontrol agents directed against various fungal phytopathogens, including *Verticillium* spp. (2). Although the mechanisms of this interaction have not yet been thoroughly investigated, the application of biopreparations and biofertilizers based on beneficial *Trichoderma* spp. strains is gaining popularity, particularly within the areas of sustainable agriculture.

The aim of the study was to investigate whether beneficial *Trichoderma* spp. fungi are effective for biocontrol of *Verticillium* sp. pathogenic fungus.

The fungi were cultivated on the PDA medium with the addition of tetracycline and streptomycin. Three *Trichoderma* strains were used in this research (G63/18, G65/18 and G78/18) and one *Verticillium* strain (G296/18). The fungal strains were isolated within EcoFruits project in the Institute of Agrophysics, Polish Academy of Sciences in Lublin. The mycelium of the tested fungi was homogenised using the inoculation loop and vortex. The suspension was then transferred onto a microscope dish and fungal spores were imaged using the Nanolive's holotomography microscope and 3D Cell Explorer. *Verticillium* sp. and *Trichoderma* sp. spores were incubated together for 20 h. During the incubation, a time-lapse observation of the spores was also performed.

The Nanolive technology provided 2D and 3D images of fungal spores. The *Trichoderma* sp. strain G63/18 was found to be aggregated on the surface of *Verticillium* sp. strain G296/18 spores and inhibited its germination. *Trichoderma* sp. strains G65/18 and G78/18 were able to gather around *Verticillium* sp. spores, but did not inhibit its germination.

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Occurrence of *Fusarium oxysporum* on popular pot plants

Magdalena Ogonowska, Joanna Barton, Jadwiga Tomalak, Zuzanna Polak, Pola Wasilewska- SKN Medyków Roślin “Armillaria”, Faculty of Life Sciences and Technology, Wrocław University of Environmental and Life Sciences

Faculty of Life Sciences and Technology, Wrocław University of Environmental and Life Sciences

104596@student.upwr.edu.pl

Key words: *Fusarium oxysporum*, ornamental plants, *Monstera* sp., *Musa* sp., *Echinocactus grusonii*

Fusarium oxysporum is a common pathogenic fungal species. The host range of this species is very wide, including plants, animals and humans. It was found in many countries, in almost all climatic zones. *F. oxysporum* infects cultivated plants such as: cotton, vegetables, fruits and cereals, as well as ornamental plants. It ranks 5th on the list of the most dangerous fungal pathogens.

During our research, *F. oxysporum* was isolated from the following ornamental plants: *Monstera deliciosa* 'Thai constellation', cactus *Echinocactus grusonii* and twice from ornamental banana. It is the most frequently isolated fungal pathogen of ornamental plants by our student organization. It caused drying and wilting of plants, starting systemic infection from the bottom of the plant, which indicates that the source of infection was in the substrate.

Reducing the occurrence of this pathogen is almost impossible due to the type of dissemination: conidia spread with wind and rain, as well as with vegetative propagation of plants. The aim of the study was to emphasize the importance and abundance of *F. oxysporum* not only as a pathogen of crop plants, but also as a common cause of diseases of ornamental plants.

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Degradation of benzo(a)pyrene by white rot fungi

Marta Pietrzak, University of Lodz

Biotechnological and Microbiological Students' Scientific Association “Bio-Mik”, University of Lodz

Department of Microbiology and Biotechnology, University of Lodz

mrtapietrzak@gmail.com

Key words: Benzo(a)pyrene, white rot fungi, degradation

Benzo(a)pyrene (BaP) belongs to polycyclic aromatic hydrocarbons and is classified as mutagenic, carcinogenic and teratogenic compounds. BaP consists of five fused aromatic rings, which give this compound high stability against biodegradation. The presence of BaP in the environment poses a threat to human and animal health, therefore it is important to look for methods to remove or degrade this compound. The efficient strategy for BaP removal is the use of microbial degradation. This strategy rests on the exploitation of the ability of microorganisms to grow by using pollutants as their source of carbon, leading to partial or complete removal of these pollutants.

White rot fungi (WRF) belong to the group of degraders, they are known for their ability to degrade chemical compounds, including BaP. Owing to a broad substrate spectrum, along with the presence of environmentally friendly degradative enzymes, they are widely used for degradation and remediation of organic pollutants such as polycyclic aromatic hydrocarbons, pharmaceutically active compounds, endocrine disruptor compounds, herbicides and pesticides. The mechanism of BaP degradation by white rot fungi is enzymatic biodegradation, in which fungal enzymes catalyze oxidation and hydrolysis reactions of a chemical compound, leading to its decomposition into simpler compounds.

Research is being carried out using the white rot fungus - *Phlebia brevispora*, which shows its ability to degrade BaP and use it to reduce the amount of this compound in the environment.

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Toxic friends - *Rhizopus microsporus* and its bacterial endosymbiont *Mycetohabitans rhizoxinica*

Zuzanna Płoszka

Mycological section of Student Naturalist Association of the Jagiellonian University

Jagiellonian University, Faculty of Biology

zuzanna.ploszka@student.uj.edu.pl

Key words: *Rhizopus*, symbiosis, rhizoxin, bacterial-fungal interactions

Fungi and bacteria live together in many different habitats. Their interactions are important for the functioning of various ecosystems. These two groups were long considered to be each other's enemies, but recent studies have shown that they can cooperate and form mutualisms. The closest type of symbiosis occurs when bacteria live inside fungal cells. One of the better studied examples of such relationship is that of *Rhizopus microsporus* and its endosymbiont *Mycetohabitans rhizoxinica*, which became a model for understanding fungal-bacterial symbioses.

Rhizopus microsporus is a soil saprotroph which can also act as a pathogen to plants and humans. Living inside the *Rhizopus* cells is *Mycetohabitans rhizoxinica*, a *Burkholderia*-related species of bacteria. The symbiont provides its host with a toxin, which enables the fungus to become a plant pathogen. Rhizoxin is the causative agent of rice seedling blight – a disease that causes significant crop losses in Asia. Research shows that the toxin also protects *R. microsporus* from predators, suggesting a possible explanation for the evolutionary origin of this symbiosis.

Certain changes in the lipid metabolism of the fungus are necessary to maintain its endosymbionts. *M. rhizoxinica* has been found to control its vertical transmission through influencing host's sexuality. Without the symbiont the host cannot reproduce asexually and its sexual reproduction is strongly impaired.

The *R. microsporus* – *M. rhizoxinica* symbiosis shows how bacteria can alter basic biological functions of their fungal hosts. Further studies of this subject may also prove important for agriculture and medicine.

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Occurrence of *Apiognomonia veneta* on *Platanus* sp. in urban greenings in Wrocław

Zuzanna Polak, Joanna Barton, Jadwiga Tomalak, Magdalena Ogonowska, Pola Wasilewska- SKN
Medyków Roślin

“Armillaria”, Faculty of Life Sciences and Technology, Wrocław University of Environmental and Life
Sciences

120073@student.upwr.edu.pl

Key words: anthracnose, *Platanus* spp., *Apiognomonia veneta*

Platanus (*Platanus* L.) are popular trees in urban greening, avenues and parks in the whole Europe. They are characterized by large (up to 25 cm wide) glossy leaves and silver-grayish bark. These trees exhibit rather intensive growth[1]. Due to the size of leaves, trees generate shade in urban areas and also reduce the effect of the urban heat island. They are mostly resistant to harsh conditions prevailing in highly urbanized areas. Despite their tendency to be large in size, they tolerate truncation well[2].

Although they show a high tolerance to unfavorable conditions, they are still threatened by plant pathogens. Some of them decrease the mechanical strength of the trees, and some cause mainly visual changes[2], which in the case of ornamental trees is an equally important issue. *Apiognomonia veneta* is listed as one of the pathogens threatening *Platanus* in urban greenings. It does not cause direct tree dieback, but significantly weakens growth and promotes their disfigurement[3]. The disease symptoms include necrosis and brown-black spots on the leaves. The spots cover the nerves and the areas of the leaf blade bordering them, the necroses intensify resulting in the death of the leaf[4].

In Wrocław municipality *Platanus* are commonly used as characteristic ornamental plants in urban greenery. The aim of the research was an assessment of the danger that anthracnose might pose to the *Platanus* tree nowadays and in the future, in Wrocław city.

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Optimization of zearalenone biotransformation by *Beauveria bassiana*

Katarzyna Prochoń¹, Sylwia Różalska²

¹Biotechnological and Microbiological Students, Scientific Association "Bio-Mik", University of Lodz

Department of Microbiology and Biotechnology, University of Lodz

k.prochon@gmail.com

Key words: zearalenone, *Beauveria bassiana*, biotransformation

Beauveria bassiana is an entomopathogenic fungus inhabiting soil environments. It can infect many species of insects in which it causes white muscardine disease. The spores of the fungus that come into contact with the body of the insect germinate, pierce the epidermis and grow inside, which leads to the death of the insect, which usually occurs within a few days. Subsequently, white mold emerges from the corpse and produces new spores.

Zearalenone is a mycotoxin produced by species of the fungus *Fusarium*. It is a toxin found worldwide in many cereal crops (e.g. corn, wheat, rice, oats). It is thermally and environmentally stable because it is insoluble in water and resistant to physical and chemical processes. Zearalenone can bind to estrogen receptors, which causes estrogenic and reproductive disorders in humans and animals.

Some entomopathogenic fungi are able to eliminate this mycotoxin by biotransformation into its metabolites. Biotransformation may depend on many factors, including the microbial medium. In this study, the biotransformation of zearalenone by *B. bassiana* in two media - Lobos and Czapek-Dox was compared, and it was assessed which of them is more optimal for the biotransformation of this mycotoxin.

After 7 days, the concentration of zearalenone on the Lobos medium was 2.31%, and on the Czapek-Dox medium was 1.39%. The obtained data suggests that zearalenone biotransformation by *Beauveria bassiana* was better on Czapek-Dox medium.

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Beneath the apple tree: exploring the biodiversity of fungal communities in soil under *Malus domestica* trees

Michał Pylak, Karolina Oszust, Jacek Panek, Klaudia Szpilska, Magdalena Frąć

Instytut Agrofizyki im. Bohdana Dobrzańskiego Polskiej Akademii Nauk, ul. Doświadczalna 4, 20-290 Lublin

k.oszust@ipan.lublin.pl

Key words: apple trees, biodiversity, soil treatments

The fungal biodiversity of soil can be greatly affected by the soil treatment and the plants present. This type of research needs to be conducted because understanding the complex relationships between plant species and soil microbial communities is critical for developing sustainable agricultural practices that promote soil health and biodiversity (Ponisio i in., 2015). Proper soil health ensures proper functioning of the environment e.g. bioavailability of micronutrients and organic matter decomposition (Tal, 2018).

Soil samples were collected from under the apple trees growing in different types of cultivated land such as bound, forest, cultivated and uncultivated orchards, gardens and gardens with animals. These represent the most common types of soil under the apple trees. DNA was isolated and subjected to next-generation sequencing (NGS). Based on this analysis alpha and beta diversity measures were calculated. The results show that different types of land cultivation significantly influence the fungal microorganisms composition in the soil.

In conclusion, this research highlights the importance of studying the biodiversity of soil fungal communities in apple orchards and beyond. By examining the effects of soil treatments on fungal diversity, this study provides a basis for developing sustainable agricultural practices that support soil health and promote biodiversity conservation.

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Effect of PBAT Microparticles on the Membrane Properties of *Trichoderma* sp.

Volha Rusetskaya, Przemysław Bernat

Department of Industrial Microbiology and Biotechnology, Faculty of Biology and Environmental Protection. University of Łódź, 90–237 Łódź, Poland.

volha.rusetskaya@edu.uni.lodz.pl

Key words: microplastic; PBAT; *Trichoderma*; phospholipids

As awareness of the impact of plastic pollution increases, interest in alternative materials is growing. One of these substances is PBAT, a biodegradable plastic (1,4-butylen-co-terephthalate). Its specific applications include food packaging film, mulching film, and other water-resistant coatings. There is evidence that even after 80 days, PBAT can be reduced by 90%. However, little is known about the effects on soil microorganisms of PBAT microparticles, which are one of the stages of plastic degradation.

Cell membranes are the primary line of resistance of the microorganism to chemical substances present in the extracellular environment. The changes observed in the integrity, permeability, and fluidity of the membrane can tell a lot about the toxicity of the compound and the defence mechanisms of the microorganism.

The aim of this study was to investigate the effects of PBAT microparticles on cell membranes of *Trichoderma* fungi.

The selected strains were cultured in soil and Sabouraud medium with microplastic. Fungal biomass was extracted by methanol. Analysis of the phospholipid profile was performed using LC-MS/MS techniques. Quantitative analysis was performed using the MRM technique. Changes in membrane permeability were assessed by fluorescence intensity of cells after incubation with propidium iodide solution.

Changes in the profile of phospholipids and fatty acids were observed during the study. Additionally, an increase in the permeability of the cell membrane was observed, which indicates the effect of PBAT microplastics on the tested fungi. The effect of the microplastic tested varies between strains, indicating differences in the susceptibility of these fungi.

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Tracking depletion and enrichment of mycobiomes in healthy and unhealthy strawberry plant and soil niches

Dominika Siegieda, Jacek Panek, Magdalena Frąc

Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin

m.frac@ipan.lublin.pl

Key words: organic strawberry, metataxonomy, SourceTracking2, soil and plant niches

The increasing popularity of organic agriculture is due to growing awareness of its environmentally-friendly practices, as evidenced by the European Union's Biodiversity Strategy for 2030^{1,2}. This strategy emphasizes expanding organic farming, reducing pesticide usage, and exploring the role of microorganisms in maintaining agricultural microbiological balance. A promising area of research is the migration of mycobiome between soil and plant niches³, which can be studied using advanced molecular biology and bioinformatics tools to gain insights into fungi movement. Understanding this process is crucial for developing effective strategies to support sustainable agriculture that reduces pesticide reliance and promotes biodiversity and soil health.

We collected the samples of soil (bulk soil, rhizosphere soil) and plant (root, shoot) niches from healthy and unhealthy organic farms of strawberry. We then performed metataxonomic sequencing of the ITS1 region with Illumina Miseq platform (2x300). We then processed the data in QIIME2⁴ and then performed SourceTracker2 analysis for each niche, comparing depletion/enrichment of fungal ASV between healthy and unhealthy plantations.

The analysis revealed, notable differences in fungal ASV depletion/enrichment between healthy and unhealthy farms, offering insights into potential mechanisms for enhancing plant health and promoting sustainable agriculture practices.

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Entomortierella parvispora - dominant *Mucoromycota* fungus in iron contaminated soil

Łukasz Słowik, Beniamin Abramczyk

Institute of Evolutionary Biology, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, ul. Żwirki i Wigury 101, 02-089 Warsaw, Poland

l.slowik@student.uw.edu.pl

Key words: Soil pollution, heavy metals, *Entomortierella parvispora*, bioremediation

Soil contamination with heavy metals is a serious problem all around the world. To restore soil properties, bioremediation is often used. Microorganisms that inhabit contaminated environments are good candidates to be used in bioremediation since they can tolerate pollution. The goal of this study was to evaluate the biodiversity of *Mucoromycota* in soil contaminated by heavy metals. 9 samples were collected from 5 sites in Poland. 3 locations were located in postindustrial areas (1 sample from Ropianka where contamination with hydrocarbons was expected, and 2 from Kolorowe Jeziorka, where elevated levels of heavy metals were expected). Control samples were collected from 2 locations near the postindustrial sites, where no contamination was expected. Concentrations of heavy metals were measured for all samples. *Mucoromycota* representatives were isolated and then classified using ITS rDNA marker sequencing and phylogenetic trees reconstructions.

Higher levels of heavy metals were found in samples from Kolorowe Jeziorka (when compared to control samples). There is no significant difference in the concentration of heavy metals between control and test samples from Ropianka. 108 stains of *Mucoromycota* were isolated including *Entomortierella*, *Linnemannia*, *Mortierella*, *Mucor*, *Podila* and *Umbelopsis* genera. *Entomortierella parvispora* was a dominating *Mucoromycota* species in soil with a high concentration of iron. Resistance to iron pollution makes *E.parvispora* a candidate for bioremediation, further research is needed to prove that.

Micromycetes as a forensic tool

Klaudyna Sychala¹, Rafał Ogórek¹, Łukasz Szleszkowski², Marcin Kadej³

¹Department of Mycology and Genetics, University of Wrocław

²Department of Forensic Medicine, Wrocław Medical University

³Department of Invertebrate Biology, Centre for Forensic Biology and Entomology, Evolution and Conservation, University of Wrocław
University and department (full name)

klaudyna.sychala@uwr.edu.pl

Key words: forensic mycology, fungi, post-mortem interval

Fungi and their structures are the main subject of forensic mycology [1, 2]. Despite its origins in 1955 [3], it is still not a well-studied science. However, the frequency of reports from the field has been increasing in recent years. This creates hopes for a wider use of mycological evidence in forensic investigations [1, 2]. The aim of this paper is to summarize the possibilities of using mycological evidence in forensic science, especially micromycetes. The main focus will be on fungi associated with human cadavers, which are part of the necrobiome - a community of species (prokaryotic and eukaryotic) associated with the decaying remains [4] that may play a role in estimating the post-mortem interval (PMI - time elapsed since death) [1, 2].

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***Neofabraea* (syn. *Pezicula*) as a biological factor causing storage disease of apples**

Klaudia Szpilska, Karolina Oszust, Agata Gryta, Jacek Panek, Michał Pylak, Magdalena Frąć

Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin

k.szpilska@ipan.lublin.pl, k.oszust@ipan.lublin.pl

Key words: *Neofabraea*, storage disease, apple, *Trichoderma*

Neofabraea sp. representatives are fungal pathogens that causes bull's eye rot (BER), which is a storage disease that affects apple quality. This disease symptoms are characteristic, and they are showing up by the development of small, circular indentations and brown lesions on the fruit surface.

The fungus infects apples during the growing season, and symptoms may not appear until the fruit is in storage. The source of infection are fungal spores that are transferred to the fruit with raindrops or by insect bites. The management of BER mainly involves the use of fungicides.

However, the use of biological control agents has been recognized as a sustainable and effective strategy. For example, *Trichoderma* sp. representatives are widely distributed in soil and has been shown to have antagonistic effects against various plant pathogens. *Trichoderma* sp. is also a prospect antagonist of phytopathogens of *Neofabraea* genus.

Recent studies have shown that *Trichoderma* sp. can not only better prevent plant diseases, but can also promote crop growth. It produces secondary metabolites and peptides that inhibit the growth of pathogens. Also, the application of *Trichoderma* sp. to apple orchards can reduce the occurrence of BER. *Trichoderma* sp. works among others by colonizing the apple surface and producing antibiotics that inhibit the growth of fungi belonging to *Neofabraea* genus.

In conclusion, *Neofabraea* sp. is a significant storage disease of apples, which can cause up to 50% of crop losses in the case of some orchards. The use of biological control agents such as *Trichoderma* sp. may provide a sustainable and effective strategy for future managing apples bull's eye rot.

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Characterization of *Graphiola phoenicis*, pathogen of *Phoenix canariensis*

Jadwiga Tomalak, Joanna Barton, Zuzanna Polak, Magdalena Ogonowska, Pola Wasilewska

SKN Medyków Roślin “*Armillaria*”, Faculty of Life Sciences and Technology, Wrocław University of Environmental and Life Sciences

120088@student.upwr.edu.pl

Key words: *Basidiomycota*, false smut, *Phoenix canariensis*

Graphiola phoenicis is an obligatory pathogen that infects plants of the genus *Phoenix* spp. Due to the appearance of etiological symptoms (small brown spots) on the leaves of its host, it is commonly called "false smut". This species is a great phytosanitary threat in places where palm trees are grown, including *Phoenix canariensis* and date palms. In Poland, this species has been observed only once on *Phoenix dactylifera* until last year when for the first time we observed it on *Phoenix canariensis*. This work is a general characterization of this pathogen based on our research.

The aim of the experiment was to investigate the pathogen - *Graphiola phoenicis* on the *Phoenix canariensis* and to assess the potential threat to ornamental potted plants in the country. The Canary Island date palms, which is a research object, were purchased in a popular supermarket, without any visible symptoms of disease. Every plant came from the same supplier. After some time, clusters of fungus spores and spots were developing on the leaves' upper side. Pictures of the plant and of the sporangia were taken. Identification was made on the basis of the morphological features of the mycelium, observed under a light microscope and binoculars.

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