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ABSZTRAKTOK/ABSTRACTS

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Agricultural and Food Microbiology, -

Bioeffector potential of Bacillus strains isolated from different Agaricus bisporus casing materials

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Pesticides are chemical compounds that are used to kill insects, rodents, fungi and weeds, so they can be used as a pest control method in agriculture, food, forestry and aquaculture. By their nature, they can accumulate and can be harmful to the environment, wildlife and humans, and significant measures are being taken across the European Union to review and possibly phase them out. In order to achieve sustainable agriculture, new practices based on biostimulants, which have less negative impact on the environment and can be more effective than synthetic agricultural chemicals, need to be developed. In this approach, one of the most important elements of sustainable agriculture is the use of biocontrol microorganisms that can reduce biotic stress, alter plant defence mechanisms and offer new methods of controlling plant pathogens and insect pests. The other is the development of treatments based on appropriate bioeffectors to help plants resist more successfully to various abiotic stress factors.

The aim of this research is to describe bacteria with biocontrol and growth-promoting potential that could be useful in the control of pathogens and serve as biostimulants for plants. In our research, 101 Bacillus strains isolated from recomposted Agaricus bisporus mushroom compost were tested for siderophore, indole-3-acetic acid, iturine, fengicin and surfactin production and subjected to pathogen confrontation tests to assess their potential future application in agriculture. Of these bacteria, B. licheniformis strain SZMC 28099 produced the highest amount of indole-3-acetic acid, while Bacillus strains SZMC 27559, 27610, 27614, 27619, 27624 and 28034 produced siderophore compounds in the range of 50 psu for two days. The strains B. subtilis SZMC 27725 and B. halotolerans SZMC 27857 excelled in lipopeptide production, while the isolate B. velezensis SZMC 27457 showed the highest inhibition against 6 pathogenic fungi. Our results support that Bacillus species may be suitable for mushroom and plant cultivation practices.

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Agricultural and Food Microbiology, -

The effect of plant growth-stimulating bacteria on the cultivation of a continuous-growing tomato variant.

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Drought is a serious natural hazard that negatively affects ecosystems, agriculture, and water resources. Recent years have seen an increase in the frequency of extreme summers in Europe, which has led to severe droughts and lower crop harvests. The use of plant growth-promoting bacteria (PGPB) has drawn interest as a potential tactic to improve plant resistance in water-limited environments in order to lessen these consequences.

In the previous year, we investigated the impacts of bacterial inoculation on the growth, yield, and nutrient composition of determinate industrial tomato plants under drought and irrigated conditions using two bacterial strains, Brevibacillus FSP5 and Kocuria FSP120. The findings demonstrated that FSP120 strain exhibited the most significant enhancements across several parameters.

Based on these promising results, this year's experiment was designed to further investigate the effects of FSP120 on an indeterminated tomato variety. The pot experiment was conducted under two different conditions: deficit irrigated (50% water capacity) and fully irrigated (100% water capacity) In the case of both irrigations, two groups were created and treated with different doses of bacterial suspension, in three parallel repetitions, with five plants per group. During the experiment, we examine the height of the plants, the number of leaves, flower buds and flowers on a weekly basis. The stomatal conductance was determined using an AP4 type portable porometer during the different phenological phases. The surface temperature of the plants was monitored using a Flir One thermal camera, and the reflectance of the leaves was determined using an ASD FieldSpec® HandHeld 2 hand-held spectroradiometer. Finally, we determine the yield averages.

The measurements and their evaluation are still ongoing.

This work was supported by the Research Excellence Programme of the Hungarian University of Agriculture and Life Sciences.

Agricultural and Food Microbiology, -

Short term plant growth promoting effects of Kocuria sp. 120 bacterial strain on tomato and maize plantlets.

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Plant growth-promoting bacteria (PGPB) act as biostimulants that boost the growth, yield, and quality of crops. Numerous studies have been conducted on various PGP genera, demonstrating their ability to enhance plants phenological and nutritional characteristics. However, some genera, such as Kocuria sp., have not been extensively studied. This research aims to determine the effects of Kocuria sp. FSP120 strain on tomato and maize plants within a phytotron system and to introduce a green fluorescent protein encoding plasmid in the bacterial cells for future root colonization tests.

The short-term effect of the strain was investigated on sweet corn and tomato plants.

Tomato seeds were sown in arable soil and peat, and four different settings were introduced: control, supplementation with nutrients or FSP120 suspension, and supplementation with the combination of nutrients and FSP120 suspension. The treatments were applied three times after germination.

For maize, all plants were grown in a peat substrate and were treated three times post-germination with either one, five, or ten times concentrated FSP120 suspension, as well as with nutrient supplementation.

In case of the tomato plants, the results showed that those grown in soil were significantly higher and had more leaves than those planted in peat. Furthermore, whether in peat or soil, all treatments had positive effect on the plant development compared to the control, combinations of FSP120 suspension with nutrients had the most beneficial impact on plant height and leaf number. From the experiment conducted on maize, we observed those plants inoculated with five and ten times concentrated FSP120 suspension presented the most favorable phenological outcomes. These included improved height, leaf count, root weight and length, as well as higher chlorophyll content in the leaves.

In order to understand the effect mechanism of the strain on the plant, we want to perform root colonization tests. Before initiating root colonization assays, it was necessary to develop a plasmid encoding kanamycin resistance and green fluorescent protein to introduce into the FSP120 competent cells. So far, the recombinant shuttle vectors have been successfully inserted into E. coli competent cells, and efforts are currently underway to introduce them into FSP120 competent cells by electroporation.

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, Agricultural and Food Microbiology, -

Assessment of the impact of gamma irradiation and varying salt concentrations on the antibiotic susceptibility of Staphylococcus aureus strains

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Staphylococcus aureus is an important Gram-positive pathogenic bacterium of food safety concern. Although it is susceptible to destruction by heat treatments such as cooking or pasteurisation, it may secrete heat-stable enterotoxins in the event of cross-contamination of food. Furthermore, among other pathogens, antibiotic-resistant S. aureus is identified as a high-priority bacterial agent in the 2024 Bacterial Priority Pathogens List (BPPL) of the World Health Organization (WHO).

Gamma irradiation represents an effective method for ensuring food safety while exerting minimal impact on food quality. In light of the growing prevalence of antibiotic resistance, this study aimed to examine the response of an MRSA and a sensitive S. aureus strain to antibiotics following exposure to environmental stressors. The stress factors employed were high salinity (osmolarity) and sublethal doses of gamma irradiation. The study evaluated alterations in antibiotic susceptibility and resistance prior to and following the application of stress, in addition to genotypic analysis to identify the presence of the mecA gene, which is associated with beta-lactam antibiotic resistance.

Two strains of S. aureus, an MRSA and an MSSA, were subjected to antibiotic susceptibility testing using the agar disk diffusion method with ten antibiotics. The MRSA was subjected to further analysis in the presence of 6% NaCl and 0.6 kGy of gamma irradiation, both individually and in combination. A control sample that had not been salted or irradiated was used for comparison purposes. The impact of the aforementioned treatments on bacterial survival and antibiotic resistance was quantified, and a PCR test was conducted to detect the presence of the mecA gene in both intra- and extracellular DNA.

It was observed that stress factors altered the behaviour of the bacteria towards the antibiotics, with some antibiotics showing increased susceptibility and others increased resistance. It is noteworthy that meropenem resistance decreased to an intermediate level with increasing NaCl concentrations (6%, 10%, 12%), and susceptibility was restored after gamma irradiation alone or combined with high salinity. The results of the polymerase chain reaction (PCR) confirmed the presence of the mecA gene across all treatments, indicating that no genetic change had occurred. The bacterial count exhibited a notable decline, particularly in the presence of irradiation.

In conclusion, the combination of high salinity and a low dose of gamma irradiation resulted in a reduction in resistance to meropenem in MRSA. The persistence of the mecA gene indicates that the observed loss of resistance may be attributed to either inhibited gene expression or altered membrane permeability. These findings indicate that the combined stressors could enhance the uptake of antibiotics, which may offer a strategy to combat antibiotic-resistant S. aureus.

Agricultural and Food Microbiology, -

Characterisation of the rhizospheric and endophytic Bacillus licheniformis strains isolated from sweet potato with yield enhancing and plant growth-promoting potential

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The primary aim of the present study was to identify bacterial isolates with yield-enhancing potential for application as biofertilizers in the cultivation of sweet potato. Therefore, endophytic and rhizospheric strains were isolated from Hungarian sweet potato to identify bacterial strains with plant growth-promoting and biocontrol potential. In total, seven Bacillus licheniformis strains were identified and subjected to detailed ecophysiological investigations. Experiments have been carried out to investigate the tolerance of selected strains to different limiting factors such as pH, temperature, and water activity, which affect survivability in various agricultural environments. The majority of tested B. licheniformis strains exhibited plant growth-promoting potential (e.g., production of indole-3-acetic acid up to 40.42 µg mL-1, production of ammonia up to 0.87 mg mL-1, phosphorus solubilising activity, siderophore production), with two strains (SZMC 27713 and SZMC 27715) demonstrating inhibitory activity (ranging between 7 and 38%) against plant pathogenic fungi occurring in sweet potato cultivation. Additionally, isolate SZMC 27715 induced accelerated germination and a significantly higher germination rate in tomato seeds in comparison to the control seeds. In a field test, it was observed that strain SZMC 27715 had a prominent yield enhancing effect in sweet potato, where a significant yield per plant increase was observed in all treatments (1.13 kg, 1.09 kg and 1.40 kg) compared to the control plants (0.92 kg). The highest yield per plant was observed when the cuttings were soaked combined with two additional foliar treatments. To our knowledge, this is the first report of the successful application of the endophytic B. licheniformis strain as a biofertilizer for yield enhancement in the cultivation of sweet potato. Based on our results, strain SZMC 27715 has potential for utilisation as a biofertilizer in sweet potato cultivation either as a standalone option or in a microbial consortium.

This work has been produced with the financial assistance of the European Union through the Interreg VI-A IPA Hungary-Serbia programme „FERTILEAVES” (grant number HUSRB/23S/11/027). The research is supported by the EKÖP-24-4-SZTE-610 University Research Scholarship Programme of the Ministry of Culture and Innovation from the source of the National Research, Development and Innovation Fund.

Agricultural and Food Microbiology, -

Antimicrobial Properties of a Grape Extract to Prevent the Use of Antibiotics in Farmed Animals

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The use of antibiotics in farming has been used for several decades as the first choice to prevent infections and promoting growth. As the demand for animal products continues to rise, so does the indiscriminate use of antibiotics in intensive farming practices. This has led to many challenges, including the emergence of antibiotic-resistant bacteria, environmental pollution, and potential risks to human health through the food chain. The need for alternatives to conventional antibiotics is increasing progressively. Polyphenols are naturally synthetized substances that have antimicrobial and antioxidant properties. This study aimed to determine the in vitro antimicrobial activity of a polyphenolic grape marc extract by the determination of MIC, IC50, and IC90 values, and to assess the possible development of increased tolerance or resistance to the polyphenolic extract in vitro. Using the broth microdilution method, we performed the antimicrobial activity and bacterial tolerance tests against Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 based, mainly on the EUCAST broth microdilution protocol. For the determination of viability of cells, we added resazurin to the wells, followed by fluorescence measurement. For the bacterial tolerance test, the surviving cells from the highest extract concentration before the MIC were taken for the next inoculation, and this was repeated until achieving 10 passes. Antimicrobial test results were as follows, MIC values for S. aureus and E. coli against the extract were for both 2.5%, the IC50 values were 1.3% and 0.94% respectively. Bacterial tolerance test results showed 5% IC90 values for both S. aureus and E. coli at the first plate, and after the 10th pass, the IC90 remained the same, indicating that no tolerance was developed for the extract during these experiments. These findings suggest that the grape extract has a potential as an alternative to conventional antibiotics in farmed animals, which could help to reduce the emergence of new antibiotic-resistant bacteria, environmental pollution, and potential risks to human health through the food chain. The potential of this extract to serve as an effective and eco-friendly alternative to antibiotics should be further tested towards developing a healthier, more resilient agricultural system.

Keywords: Minimal Inhibitory Concentration, antimicrobial activity, bacterial tolerance test, grape extract.

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Agricultural and Food Microbiology, -

Application of plant growth promoting bacteria from arid areas in tomato cultivation

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The Hungarian agricultural environment is transforming via climate change .Drought is a major challenge for plant growth especially in intensive agricultural areas. Bacteria that stimulate plant growth are often isolated from extreme environments such as arid regions and these bacteria are able to increase the survival of other types of plants during the dry period, not just those from which they have been originated.

The objective of this study is to investigate whether the isolated bacteria from arid open and closed sand steppes with plant growth-stimulating properties can improve the growth of tomato under normal and drought stress conditions.

A total of 149 strains were isolated from the sandy grasslands and the selected nonpathogenic 48 strains were screened for plant growth promoting (PGP) traits, such as: osmotic stress tolerance, indole-3-acetic acid, exopolysaccharide, siderophore, and 1-aminocyclopropane-1-carboxylate deaminase production and phosphate solubilization. The strains with the best properties were tested in a phytotron system on tomato seedlings in short-term experiments.

The strains with the best properties were tested in a phytotron system on tomato seedlings in short-term experiments. Based on the phytotron results, the long-term effects of two strains (Kocuria sp. FSP120 and Brevibacillus sp. FSP5 strains) were investigated in greenhouse experiments, where two different irrigation (100% and deficit) settings were introduced. According to the results, the FSP120 strain had a positive effect on the average height of the plants, the number of leaves, the number of flower buds, flowers and fruits. In the 100% irrigated area, the yield average of the FSP120 stand was 15% higher compared to the control group. In the deficit-irrigated stand, the average yield of FSP5 threated plants was 26%, while the average yield of FSP120 treatment was 17% higher than the control group. As a result of the bacterial inoculation, we also observed an increase in the carotenoid content of the fruits.

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Agricultural and Food Microbiology, -

Screening of green microalgae based on antibacterial activity and in-vitro testing on artificially infected fruit flowers

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In recent years, alternative and biological defense methods have gained prominence in combating plant diseases. One potential source of biological pesticides is microalgae, whose excellent adaptability is supported by a series of metabolites produced to survive extreme conditions. There are many microalgae, e.g. Chlorella sp., Scenedesmus sp. with proven antibacterial effects presumably associated with polyphenols, alkaloids, terpenes, polysaccharides, fatty acids, sterols, lactones, and proteins.

The purpose of our study was to estimate the putative inhibition effect of metabolites produced by selected algae on widely occurring facultative human pathogens (E. coli NCAIM: B.01992, S. aureus NCAIM: B.01055 and P. aeruginosa NCAIM: B.01057), the fire blight plant pathogen (E. amylovora NCAIM: B.01975.) and plant pathogen causing bacterial canker of tomato and potato blight C. michiganensis subsp. michiganensis M-764/2020.

First, we performed the biological screening of organic solvent extracts of single-cell freshwater green microalgal cultures originated from the collection of Albitech Biotechnology Ltd. Two different organic solvents – ethanol and diethyl-ether – were used to create the extracts from the lyophilised biomass. The antibacterial effect of the extracts was determined using the agar gel diffusion method. The minimum inhibitory concentration (MIC) was also measured.

We have confirmed the antibacterial effects of four single-cell freshwater microalgae strains against facultative pathogenic bacteria in-vitro. The E. coli strain was the least susceptible to treatments and S. aureus was the most sensitive. Both growth controls yielded the expected results, whereas the tested bacteria displayed sensitivity to streptomycin and exhibited positive inhibitory effect versus the algal extracts. Consequently, we determined the MIC of our algal extracts against C. michiganensis strain M-764, and the highest concentration tested 1.12 mg /ml clearly inhibited the growth of the bacterial culture. Using artificially infected various pear blossoms we were able to detect significantly reduced extent of E. amylovora infection due to antibacterial algal extracts.

Keywords: microalgae, antibacterial, agar diffusion, microdilution

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Agricultural and Food Microbiology, -

A comprehensive analysis of the microbial community present within noble rot grape berries.

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The process of the noble rot of grapes, caused by the fungus Botrytis cinerea, is a highly specific phenomenon that occurs under the optimal weather conditions in specific wine-producing regions around the wrold. The Tokaj-Hegyalja wine region of Hungary offers optimal conditions for the production of noble rot berries infected by Botrytis cinerea, locally known as "aszú" grapes. The grapes are typically harvested at the end of October or the beginning of November, allowing for a considerable interval between the final plant protection treatment and the onset of fruit development. During this period, a variety of fungi and bacteria present in the environment colonise the grapes, which can have a beneficial or detrimental impact on the fruit's health and, consequently, on the chemical and biological properties of the wine produced from it.

In the present study, we have employed DNA amplicon sequencing to analyse the bacterial communities present in different stages of aszú berries and grey rot berries. This has enabled us to identify the most important elements of the bacterial community on the berry and to ascertain their potential role in the various microbial processes. The bacterial genera identified may have a negative impact on the fermentation process of grape berry wine, but conversely, there are also genera that could positively influence the lifespan of the fruit during storage or the wine fermentation process. Furthermore, our research examined the impact of disparate cultivation sites on the structure of the bacterial community, elucidating which species are ubiquitous and which are site-specific. The resulting bacterial community data were also analysed using a network analysis method, which enabled us to identify groups within the community that are presumed to perform specific functions, with their occurrence clearly linked to either the type of rot or the site.

Agricultural and Food Microbiology, -

The role of key fungal community members on grape berries affected by Botrytis cinerea infection, which is commonly referred to as noble rot.

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The fungus Botrytis cinerea infects grapes, causing noble rot under specific weather conditions. This process gives rise to a berry that displays distinctive physical, chemical and biological characteristics, which find application in the production of premium wines in a number of globally significant viticultural regions. The objective of our research was to investigate the role of the main living filamentous and yeast fungal genera members on the grapes under these conditions. These included Alternaria, Aureobasidium, Epicoccum and Rhodotorula. RNA extraction was carried out on samples collected in Tokaj, Hungary. Metatranscriptomic sequencing was then performed on the extracted RNA, and the resulting

data were analysed using pathway enrichment and network analysis methods. In addition to Botrytis cinerea, our hypothesis was that several other microbes are also present and affect the final product's chemical composition. Our findings indicate that members of the most significant fungal community on the noble rotten grapes exhibit a highly active metabolism, which plays a pivotal role in maintaining the dynamic equilibrium of the evolving microbial community and the chemical composition of the final product, consequently influencing the properties of the wine obtained. Furthermore, we have conducted a comprehensive investigation into the potential impact of the aforementioned fungi on the aroma of wines produced from noble rotten grapes.

Agricultural and Food Microbiology, -

Fungal community of different Tokaj vineyards those related to noble rot grapevine production

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Botrytis cinerea, a well-documented pathogen of grapes, has been observed to affect over 1200 different plant species, with the most notable consequence being the development of grey rot in grapes. In specific microclimatic conditions, however, it can result in a distinctive and advantageous process known as noble rot. An illustrative example of this phenomenon can be observed in the Tokaj wine region of Hungary, where aszú wines are produced. In addition to B. cinerea, other fungi and yeasts play a role in the secondary metabolism of grapes, influencing the organoleptic and analytical properties of wines with noble rot. In the present study, sequences were extracted from ITS2-based DNA amplicons derived from healthy, noble rot, and gray rot berries obtained from three distinct vineyards of the Furmint variety in the Tokaj wine region. The fungal communities of the collected samples were analysed based on metabarcoding data, which was then used to characterise the vineyards. The findings of our study provide a foundation for future research on the functional role of noble rot in wine production and its impact on wine characteristics.

Agricultural and Food Microbiology, -

Spatial distribution of fungicide-resistant Botrytis cinerea mutations in the wine regions of Tokaj and Eger, according to the local pest management strategies used

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Botrytis cinerea is a fungal pathogen with a particularly wide host plant spectrum, causing significant economic damage in vineyards on an annual basis. It is responsible for serious yield losses and is therefore a major concern for grape growers. As a pervasive, polyphagous fungal pathogen with both saprophytic and parasitic characteristics. The sequential use of active substances belonging to the same chemical family to protect vineyards can result in an increase in fungal chemical resistance, as evidenced by the enrichment of point mutations in

the genomic regions coding for proteins involved in the mechanism of action of different pesticides. The objective of our study was to compare the sensitivity of B. cinerea populations in two wine regions with different pest management practices. The Tokaj region is characterised by the presence of B. cinerea, which is necessary for the production of noble rot wines. In contrast, the adjacent Eger region aims to achieve total protection against B. cinerea. Our study represents the inaugural Hungarian report concerning specific previously studied resistance mutations in the ERG27 and SDHB protein-encoding genes. We identified point mutations in the ERG27 transmembrane domain that have yet to be previously described but may potentially impact the emergence of resistance to select fungicides. Our findings demonstrate that the B. cinerea population of the Northern Hungary region exhibits a consistent pattern of increased fenhexamid resistance.

Agricultural and Food Microbiology, -

Effect of post-harvest diseases on purple and anthocyanin-less pepper breeding lines

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Capsicum species contain numerous phytonutrients (flavonoids, anthocyanins, vitamins, etc.) with antioxidant and antimicrobial properties that have positive effects on the human health. From a commercial perspective, shelf-life is an important characteristic, which depends on the internal composition of the produce and its level of ripeness. It is estimated that post-harvest losses account for 25-35% of total production. Studies carried out with tomatoes have shown that anthocyanins not only slowed down the over-ripening process but also made the purple fruits more resistant to one of the most significant storage pathogens, B. cinerea. Nowadays, more and more purple pepper varieties are available on the market, but little research is available on this subject. Therefore, in our work, we are investigating the resistance of purple and anthocyanin-less breeding lines to storage pathogens.

For the experiments, near-isogenic lines from Gábor Csilléry's mutant collection were applied, which fruits are green or purple at economic maturity and turn red at biological maturity. The fruits were collected at the same phenophase, were surface sterilized and placed in sterile containers. The infections were carried out with Fusarium culmorum and Botrytis cinerea, previously set to a spore count of 4.5 × 105 spores/ml. To do this, a 5 mm wide and 2 mm deep wound was made on the fruits, and 5 µl of the fungal suspension was pipetted into the wound. The infected purple, green, and red fruits were kept at 25°C with high humidity. The phenotypes of the infections were recorded on days 7 and 13 and analysed with ImageJ. For quantitative determination of the infections, qPCR was performed. For this, the fruits were lyophilized and then powdered, followed by DNA isolation and qPCR. Fruits were also used for growth tests of the pathogens. Differently coloured fruits were homogenized then sterilized after centrifugation. PDA medium was supplemented with 50% of the resulting extract. Fungi growth were measured for a duration of 3 days.

Significant differences were scored between the ecomomical and biological ripeness, where the fruits being in latter phenophase proved to be more resistant against the pathogens. In

contrast to experiments conducted with tomatoes, purple peppers were not shown to be more resistant to either Fusarium or Botrytis infection. The green and purple peppers at economic maturity did not show significant differences in their infection phenotypes. In terms of quantitative determination, significantly higher infection rates were observed in purple peppers compared to green peppers for Fusarium. As stated, biologically mature fruits differed significantly from those of economically mature peppers in terms of their infection phenotypes. One exception was found with Botrytis, where no significant difference in infection levels was observed between green fruits and those turning red from purple. The growth tests on the PDA medium showed similar results ie., the extract of the biological ripe fruits exerted greater effects on the pathogens.

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The future of walnut production challenged by the walnut husk fly and microbial pathogens

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Walnut is an economically important hardwood tree species cultivated worldwide. According to the Hungarian Chamber of Agriculture, walnut is the most significant shell fruit in Hungary, with an annual production ranging from 7,000 to 10,000 tons, and the area of mature orchards covering approximately 5,000 to 6,000 hectares in 2022. Due to its exceptional quality, Hungarian walnuts are in high demand in the Hungarian and European markets. However, due to cultivation and plant health issues related with climate change, walnut production and quality has been declining year by year. In recent years, the walnut husk fly (Rhagoletis completa), native to North America, has appeared and spread significantly in Europe, including Hungary. Its larvae develop in the walnut husk, significantly reducing the quality and quantity of the crop, which can also be attributed to potentially unknown microbial pathogens spread by the walnut husk fly. The decline of walnut trees can be attributed to various bacteria (e.g., Xanthomonas species) and fungi (e.g., Phytophthora, Armillaria and Ophiognomonia species), which are increasingly capable of damaging the walnut trees when their resistance decreases. Based on currently observed trends, walnut trees may disappear from gardens within a few years. Currently, the protection of walnut trees primarily involves chemical solutions, which may be effective against the walnut husk fly and other pathogens. However, the residues of these chemicals can enter the food chain, posing long-term environmental and health risks. Increasing concerns about the negative impacts of chemical control are prompting researchers to develop more environmentally friendly solutions, such as biological control methods.

Our research aims to analyze the diversity of walnut tree-associated microorganisms collected in Hungary. In addition, we aim to unravel the role of walnut husk flies as possible vector organisms of microbial walnut diseases by metagenomic methods, and to analyze the microbial components involved in the infection process using metabarcode markers. During our investigations we collect soil and phyllosphere samples, as well as larvae of walnut husk flies from walnut plantations that were severely and less affected by walnut pathogens. Besides walnut pathogenic microorganisms, various potential microbial biocontrol agents (e.g., Trichoderma, Bacillus, Pseudomonas) are also isolated from the samples, which is followed by strain identification and detailed characterization.

Our results will provide an essential base for developing environmentally friendly and sustainable solutions to protect walnut trees, thereby contributing to the long-term preservation of walnut production in Hungary.

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Production of bioactive extracts from sorghum grain residues using lactic acid bacteria-based fermentation

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Cereals and whole grain food products are essential components of our diet, largely due to their rich content of health-promoting compounds, including fibers, vitamins, and phenolic molecules. Sorghum, in particular, has gained increasing importance in the gluten-free diets. Natural phenolics are extensively studied and recognized for their antimicrobial and other beneficial properties. However, the majority of phenolics in sorghum grain are present as phenolic-esters and/or phenolic-glycosides, which limits their bioavailability. Lactic acid bacteria (LAB) are known for their positive effects on the human health, such as the production of vitamins, antimicrobials, and antioxidants, as well as their ability to improve intestinal microbial balance. These bacteria can also produce esterolytic and cellulolytic enzymes, which may enhance the release of bioactive phenolics from their bounded form. Moreover, the growth and/or activity of probiotics, including LAB, can be stimulated by prebiotic and bifidogenic food ingredients present also in cereal substances.

The aim of this work was to examine the effect of LAB-based fermentation on the phenolic content and antioxidant activity of sorghum grain residues, using the Lactobacillus acidophilus LA5 as fermenting microorganism. Furthermore, effect of fermentation on the viable count of the L. acidophilus was also monitored. Cultures were incubated for 24, 48 and 72 hours under oxygen depleted condition. After fermentation, colony forming unit (CFU) was determined through spread-plate method using MRS agar plates. The total phenolic content (TPC), antioxidant activity and enzyme activity values of purified ferments were measured by spectrophotometric assays.

Fermentation on grounded sorghum grain had a positive effect on the growth of L. acidophilus. Namely, the sorghum supplemented growth environment caused an 8-fold increase in the CFU after both 24 and 48 hours of incubation compared to the cereal-free system. The beta-glucosidase and cellobiohydrolase enzyme activities measured in the cell free supernatant were also increased during the fermentation. The level of TPC and antioxidant activity in fermentation systems varied depending on the duration of incubation and on the presence of L. acidophilus. Anyway, addition of sorghum grain residues supported the L. acidophilus growth, resulting in extracts with considerable phenolic content and antioxidant capacity.

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Agricultural and Food Microbiology, -

Interactions between Trichoderma and Armillaria mediated by volatile organic compounds in the light of biocontrol strategies

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The interaction between Trichoderma atroviride and Armillaria ostoyae, involving volatile organic compounds (VOCs), is a prime example of the complex network of biochemical signalling and ecological interactions between fungi. Our current focus is on understanding the basis of VOC production in each species and the VOC-related background of using T. atroviride as a biocontrol agent (BCA) against the tree root rot pathogen A. ostoyae. We employ a dual culture assay, gene expression profiling, and GC-MS analysis to elucidate the molecular communication between A. ostoyae and T. atroviride through VOC-mediated interactions.

The focus is on identifying regulatory pathways that control VOC production based on the sensing of VOCs from the other partner by both fungi through differential gene expression analyses. T. atroviride produces a range of VOCs including potent irritants such as 6-pentyl-2H-pyran-2-one (6PP). These compounds induce the upregulation of gene expression profiles associated with chemical defence mechanisms and possibly necrosis in Armillaria ostoyae. Interestingly, a series of VOCs produced by Armillaria in the control setup are disappearing during the interaction with Trichoderma, suggesting that the Trichoderma-induced apoptotic process in Armillaria may already start before physical interaction of the two fungi.

This study aims to provide insight into the complex molecular dialogue between A. ostoyae and T. atroviride and to identify potential VOC targets for fungal ecology and biocontrol strategies. The results obtained will provide a basis for future research to understand fungal-fungal interactions at the level of VOCs for use in the development of agriculture, forestry, and environmental sustainability.

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Environmental Microbiology and Biotechnology, -

Effect of a Ligilactobacillus salivarius SK45 on aflatoxin production by Aspergillus flavus Zt41

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Mycotoxins are secondary metabolites of filamentous fungi and pose very significant human health and feed safety problems. Of the mycotoxins, aflatoxin stands out as the most potent carcinogenic compound of natural origin. In our country, for climatic reasons, AFB1-producing strains of aspergilli appeared only in the early 2010s, leading to the 2012 aflatoxin M problem affecting the dairy industry through AFB1 contamination of maize silage used for the feeding of cows. This contamination comes predominantly from AFB1 containing maize, but moulds on the sides of industrial silos, from areas in contact with the air, are capable of providing additional toxin loads.

In our work, we investigated the ability of lactic acid bacteria to antagonise toxin-producing moulds and to reduce toxin production. For the experiments, we used the Aspergillus flavus Zt41 strain, which was the first AFB1 hyperproducer strain to be isolated in Hungary in 2010, capable of producing 50 ppm toxin on corn grits. As a first step, 92 different strains of lactic acid bacteria were tested for antagonism on agar plates against the mould. The growth of A. flavus Zt41 was best inhibited by the strain SK45 isolated from cassowary (Casuarius casuarius). According to molecular taxonomic identification (16S rDNA analysis), strain SK6 belongs to the species Ligilactobacillus salivarius. Ligilactobacillus is a genus of lactic acid bacteria associated with vertebrate hosts, formed through the 2020 division of the Lactobacillus genus.

The effect of L. salivarius SK45 on the production of AFB1 by A. flavus Zt41 was investigated in 200-litre silos on silage maize substrate. In addition to the control, we had one silo inoculated with A. flavus Zt41 and one silo inoculated with Zt41 and the L. salivarius SK45 strain. The silos were not fully enclosed, modelling conditions at the edge of industrial silos. During the experiment, AFB1 content was measured for 2 months. At the endpoint, 176 ppb of AFB1 was produced in the mould-only setting and 105 ppb in the system inoculated with L. salivarius SK45.

AFB1 production is the result of the coordinated action of more than 20 genes. Of these, data are available on the changes in the expression of the regulatory genes aflR, aflP (omtA), aflU (cypA), aflM (ver-1) and aflQ (ordA) in response to variations in water activity, temperature, light and pH. In our experiment, maize micro-silos were assembled and inoculated with A. flavus Zt41 and Zt41+ L. salivarius strains and incubated for 21 days at 30 ºC and 37 ºC in the dark. On days 12, 16, and 21 of incubation, the expression of aflR, aflP, aflU, aflM and aflQ genes was monitored by qRT-PCR. The final AFB1 level was then measured by HPLC analysis. AFB1 levels were significantly reduced by more than half (1.05 ppm-0.39 ppm; 1.32

ppm-0.56 ppm) in systems incubated at 30 ºC and 37 ºC. This correlated well with the significantly reduced expression of aflU and aflQ in the 30 ºC and aflR, aflP and aflM in the 37 ºC setting among the AFB1 synthesis genes tested.

Our experiments suggest that L. salivarius strain SK45 may be a promising bioagent for lowering aflatoxin contamination of air-exposed side-regions of maize silage.

Agricultural and Food Microbiology, -

How Arbuscular Mycorrhizal Fungus inoculation influence the attack of Cucumber Mosaic Virus and powdery mildew infection in tomato plants

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Arbuscular mycorrhizal fungi (AMF) can help plants manage both abiotic and biotic stresses, such as drought, salinity, heavy metal toxicity, and pathogen infections. By forming a symbiotic relationship with plant roots, AMF improve water and nutrient uptake and regulate stress-related genes, antioxidants, and hormones, enhancing plant resilience. Although the role of AMF in abiotic stress tolerance is well-known, more research is needed to understand their impact on biotic stress, which could benefit sustainable agriculture and environmental management. Our study aims to shed light on how mycorrhizal interaction influences the stress response of tomato plants during viral or fungal infections.

Tomato (Lycopersicon esculentum Mill.) seeds were germinated and grown in a sterilized substrate under controlled greenhouse conditions. Half of the plants were inoculated with Funneliformis mosseae (AMF+) using standard inoculation procedures, while the other half were left uninoculated as controls (AMF-). After 6 weeks, the tomato plants (AMF+ and AMF-) were exposed to either powdery mildew or Cucumber Mosaic Virus infection to simulate biotic stress conditions. Following 4 weeks of growth, the plants were harvested, and their physiological parameters were assessed. Additionally, changes in the expression of the phenylalanine ammonia-lyase (PAL) gene, a key component of the salicylic acid pathway activated in response to biotrophic pathogens, were analyzed.

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Agricultural and Food Microbiology, -

Native Shrubs and Fruit Trees as Potential Inoculum Sources in Viticulture

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Viticulture is one of the leading agricultural sectors in Hungary. Vineyards are often embedded in a landscape of fruit orchards and semi-natural vegetation of grasslands and shrublands, often dominated by Rosaceae shrubs, such as dogrose (*Rosa canina*) and blackthorn (*Prunus spinosa*). However the possible impact of the surrounding vegetation on grapevine (*Vitis vinifera*) health is scarcely known. We compared plant pathogenic fungal communities in wood and leaf samples of grapevine, apricot (*Prunus armeniaca*), pear (*Pyrus communis*), dogrose, and blackthorn. Samples were collected in March, June and September in the Eger wine region. ITS2 rDNA metabarcoding data were generated using Illumina MiSeq and were analyzed with *dada2* in R, while taxonomic characterization was done in *usearch* using the latest (2023) release of the UNITE reference database. Plant pathogenic fungi were selected from the dataset based on functional guild assignment using the FungiTraits database. Because leaf and wood communities differed greatly, we analyzed them separately. Dominant pathogenic genera, among others, included *Phyllosticta*, *Alternaria*, and *Ascochyta* in leaf and *Seimatosporium*, *Phaeomoniella*, and *Herpotrichia* in wood. We found that leaf communities were characterized by strong seasonality (26% of compositional variance) and non-significant host effect, while the opposite was true for wood communities, with host explaining 14% of variance. In addition, we observed compositional overlap of plant pathogenic fungi among grapevine, fruit trees and native rosid shrubs, indicating several pathogenic fungi are known to infect both grapevine and rosid fruit trees and that surrounding vegetation can serve as potential sources of inoculum.

Agricultural and Food Microbiology, -

Effect of long-term storage on the microbial community of sperm in four different variants of goldfish (Carassius auratus)

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The sperm microbiome is relatively understudied in fish biology, with limited literature on goldfish. Short- and long-term sperm storage offers a means to avoid reproductive challenges like asynchronous gamete production and declining sperm quality, while also preserving high-value gametes and facilitating sperm transport. However, sperm quality and viability of stored sperm are influenced by several factors such as male variability, storage conditions, and

biochemical processes within the sperm. Bacterial and viral pathogens can survive cryopreservation, therefore bacterial overgrowth can also decrease cell viability, reduce fertility, and influence embryo development. Besides other internal and external sources, liquid nitrogen vapor also poses a microbial contamination risk. While antibiotics are recommended during storage to inhibit microbial growth, the literature does not provide information on the bacterial composition of sperm samples. The aim of this study is to characterize the sperm microbial community of four variants of goldfish (common goldfish, shubunkin, black moor, oranda), which may contribute to the targeted treatment of samples during storage. Bacterial communities from fresh and cryopreserved (-196 °C)/thawed sperm were determined using tryptic soy agar (TSA) medium. Culture tests were performed to determine the colony-forming units (CFU mL-1) of the bacteria present. Species-level identification of typical colonies was conducted using 16S rDNA sequencing. In fresh goldfish sperm samples, 3-5 species were identified per variant, with members of the Aeromonas and Vibrio genus found across all variants. While there was no significant increase in colony numbers after thawing, species composition became more diverse. Four species were isolated from the extender applied for cryopreservation, two of which were also present in the thawed samples. Nevertheless, the change in diversity remained significant even when these two species were taken into account. Our work highlights the impact of cryopreservation technology on the microbial community associated with fish sperm. The effect of the bacterial species identified after thawing on fertilization and embryo development has not yet been investigated. This work was supported by the Research Excellence Programme and the Flagship Research Groups Programme of the Hungarian University of Agriculture and Life Sciences. Our work was also funded by the project 2022-1.2.6-TÉT-IPARI-TR-2022-00002 within the framework of the National Research, Development and Innovation Fund announced by the Ministry of Culture and Innovation. The experiments were also supported by the ÚNKP-23-3 New National Excellence Program of the Ministry of Culture and Innovation from the source of the National Research, Development and Innovation Fund.

Agricultural and Food Microbiology, -

SCREENING FOR ACQUIRED ANTIBIOTIC RESISTANT GENES IN THE GUT MICROBIOTA OF AFRICAN CATFISH (Clarias gariepinus x *Heterobranchus longifilis*) JUVENILES IN HUNGARY

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Antibiotic-resistant bacteria have emerged and spread around the world as a result of the extensive use of antibiotics as a selection factor, raising concerns about global food safety. Our research aims to investigate potential pathways of transmission for acquired antibiotic resistance genes (ARGs) and resistant bacterial strains, as well as to analyze potential epidemiological interactions between antibiotic-resistant bacteria of domestic animal, wild animal, or of environmental origin from a One Health perspective. Shotgun metagenomic

sequencing of gDNA purified from intestinal content samples of farmed African catfish hybrids (Clarias gariepinus × Heterobranchus longifilis) was carried out on Illumina NovaSeq 6000 platform. The African catfish intestinal metagenomic contigs harbored acquired ARGs of several antibiotic classes, including the sulfonamides, aminoglycosides, quinolones, and tetracyclines. The detected acquired ARGs were tetA(P), tetB(P), tet(L), lnu(C), erm(35), ermX, ant(3'')-Ia (aadA9), qnrD1, sul1, dfr(G), and fosB. The African catfish intestinal acquired resistome primarily consisted of ARGs reported from catfish species in other countries as well. The tetracycline class was one of the dominant ARG classes in both African catfish and common carp, according to comparison with ARGs found in common carp intestine from the same geographic region in Hungary. Additionally, both fish species featured a sul1 gene and a qnr-type acquired quinolone resistance determinant (qnrS2 in carp or qnrD1 in catfish). The 3'-Conserved Sequence (3'-CS) of class 1 integrons, which are common in antibiotic-resistant strains of Aeromonas spp., Pseudomonas spp., and other bacteria of aquatic habitats also includes a sul1 dihydropteroate synthase gene. Overall, the distinct gut microbiota compositions of African catfish and common carp were associated with different acquired resistome profiles. To the best of our knowledge, metagenomic techniques have not previously been used in Hungary to investigate the acquired resistome of farmed African catfish.

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Agricultural and Food Microbiology, -

Transcriptomic Insights into Mycorrhizal Tomato Plants: Response to Heat Stress

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Heat stress significantly limits plant growth and yield, leading to substantial financial losses. Consequently, it is crucial to deepen our understanding of the mechanisms that either mitigate or enhance plant tolerance to heat stress. We conducted an experiment where tomato plants inoculated with Septoglomus constrictum were exposed to a heat shock and utilized an Illumina sequencing-based transcriptomic approach followed by a comprehensive bioinformatic analysis to explore the interaction between the arbuscular mycorrhizal fungus and tomato under heat stress.

Based on the stress markers observed (H2O2 and MDA), our findings indicate that, compared to the control group, mycorrhizal plants exhibited better resilience under heat stress. This phenomenon was accompanied by the expression change of several enzymes participating in the mediation of the redox state of target plant. The lower levels of H2O2 parallels well to the downregulation of superoxide dismutase (SOD) genes in mycorrhizal treatments and the higher expression of peroxidases and glutathione S-transferase genes. The presence of

arbuscular mycorrhizal fungus influenced genes involved in the general stress responses, heat stress responses, metabolic activities, and transport processes under stress conditions. Furthermore, we observed transcriptional changes related to jasmonate and abscisic acid phytohormones playing crucial roles for both mycorrhizal development and stress response. Mycorrhizal plants, compared to their non-mycorrhizal counterparts, appear to utilize additional mechanisms to enhance stress tolerance.

Our preliminary results and dataset provide a valuable foundation for future research aimed at expanding our understanding of symbiotic interactions during high-temperature stress.

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Agricultural and Food Microbiology, -

Effect of T-2 toxin on *Agaricus* and *Pleurotus* production

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Due to climate change, there is an increasing risk of potential mycotoxin contamination of wheat straw, which is one of the main raw materials in white button (*Agaricus bisporus*) and oyster mushroom (*Pleurotus ostreatus*) production.

As currently not too much detail is known about their impact. Our main aim was to test the effect of an artificial T-2-toxin contamination on *Agaricus* and *Pleurotus* production.

T-2-toxin concentrations were measured via UHPLC-MS/MS in the mushroom substrate (during colonisation and fruiting body production) and in the fruiting bodies of *A*. *bisporus* and *P*. *ostreatus*. Lignocellulose degrading enzymatic activities and mushroom yields were compared to uncontaminated controls. Additionally microbial community composition was monitored via amplicon sequencing of bacterial and fungal barcodes.

Concentrations of T-2-toxin in the substrate were decreasing very sharply during the approximately one-month long experiment, and its concentration in the fruiting bodies were under the detection limit. Only HT-2 toxin, a degradation product of T-2, could be detected in the fruiting bodies lower than the maximum permissible limit. The toxin had no significant effect on enzymatic activities and on microbial community composition.

To understand better the fate of T-2 toxin a side experiment was created to check the detectability of T-2 toxin in mushroom substrates right after artificial contamination during a few days' time-frame.

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Agricultural and Food Microbiology, -

Investigation of bacterial strains isolated from carp (Cyprinus carpio) gut and gamete samples to develop a probiotic feed supplement

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In Hungary, common carp (Cyprinus carpio) plays a dominant role in pond fish farming. The reproductive success of this species is difficult to predict and in many cases very low fecundity can be observed for a given stock, which reduces the efficiency of fish production.In addition to environmental factors, a dysbiosis in microbiological balance may play a significant role, leading to a drop in reproductive biological parameters. The use of probiotics in fish feed is an area of growing scientific interest. Our experiments aimed at pre-selecting microorganisms with potential probiotic activity from carp (Cyprinus carpio) intestine and gamete samples, according to EFSA (European Food Safety Authority) recommendations. The scientific work involved sampling and targeted microbiological culture testing of 15 male and 15 female carp individuals from a local fish farm using Tryptone Soy Agar (TSA) and selective De Man-Rogosa-Shape (MRS) media. During sampling, 125 isolates were collected. The 20 most promising Lactococcus raffinolactis, Lactococcus chungangensis, Lactococcus lactis, Lactobacillus curvatus, Lactobacillus plantarum, Liquorilactobacillus hordei strains were subjected to detailed studies. As a first step, the viability of the strains was investigated by monitoring the optical density of bacterial suspensions inoculated in liquid culture (tryptone-soy nutrient solution) using a spectrophotometer (600 nm wavelength). Further studies on the uniformly and intensively growing strains were performed to determine the pathogenic inhibition capacity of the isolates against Escherichia coli, Pseudomonas aeruginosa and Aeromonas sp. strains. The final step of our study was the determination of the antibiotic resistance of probiotic candidate strains by the Minimum Inhibitory Concentration (MIC) test for 10 antibiotic agents. These preliminary assays can narrow down the number of isolates to the effective and safe bacterial strains that may provide an opportunity for the development of a probiotic feed supplement formulation specifically for common carp in the future.

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Molecular and Clinical Microbiology , -

Phenol-Soluble Modulin alpha3 Induces Autophagy in the HaCaT Cell Line

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Staphylococci exhibit exceptional adaptive potential, mainly due to their multifactorial virulence. Staphylococci possess structural components, secreted enzymes and various exotoxins, promoting colonization and establishment of various infections. Staphylococcus aureus and coagulase-negative staphylococci produce some pore-forming toxins, such as phenol-soluble modulins (PSMs). PSMs exert pro-inflammatory, antimicrobial, pro-apoptotic and lytic actions. Since the effects of PSMs on autophagy have not yet been investigated, we evaluated the autophagic activity in the HaCaT keratinocyte cell line treated with recombinant PSMalpha3. The levels of LC3B-II and autophagic flux were determined by western blotting. The intracellular localization of LC3B and Beclin-1 was measured by immunofluorescence assay. Cytoplasmic acidification was investigated by acridine orange staining. The ultrastructural features of control and PSMalpha3-treated cells were evaluated via transmission electron microscopy. The activation levels of signaling pathways implicated in autophagy regulation were investigated by using a phosphokinase array and western blot analysis. The results showed that PSMalpha3 elicited a significant increase in the level of LC3B-II. The calculation of the formation, degradation, and net turnover ratios indicated that PSMalpha3 significantly increased the formation rates, while the degradation rates did not change. This toxin, decreased the net turnover ratio significantly, reflecting a different relative rate of autophagosome formation and degradation. The average numbers of LC3B-positive vacuoles per cell were significantly higher in the cultures treated with PSMalpha3 than in the control cultures. Furthermore, PSMalpha3 promoted the development of acidic vesicular organelles. The phospho-Akt1/2/3 (T308 and S473), and phospho-mTOR (S2448) levels were decreased, whereas the phospho-Erk1/2 (T202/Y204 and T185/Y187) level was increased in PSMalpha3-treated cells. Thus this stapylococcal pore-forming toxin causes a pro-autophagic shift in signaling that regulates autophagy. Transmission electron microscopy showed that, init

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Epidemiological and functional studies of the β-lactam/β-lactamase combination resistance mechanisms in Bacteroides species

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Background: β-lactam/β-lactamase combination resistance is increasing among anaerobic bacteria, mainly in Bacteroides. In aerobic counterparts β-lactam/β-lactamase combination resistance usually arises by amino acid changes for Class A β-lactamases. In Bacteroides there are some β-lactamases (CfiA, CfxA and PbbA) that can mediate β-lactam/β-lactamase combination resistance. In this study we aimed to investigate in more detail what factor (which genes with what amino acid substitutions and gene activations) codes for β-lactamase combination resistance among Bacteroides strains.

Materials/methods: 48 B. fragilis group isolates were selected whose amoxicillin/clavulanate (fixed ratio) MICs has been already recorded by agar dilution in an earlier antibiotic susceptibility study. These were enriched by obtaining MICs by a fixed inhibitor concentration gradient method. We detected the cepA, cfxA, cfiA and pbbA genes by RT-PCR and sequenced and detected the 5' regions of the cfxA genes. We did conjugation experiments to transfer the resistance phenotype of some selected strains to a susceptible host.

Results: None of the strains carried the cfiA or the pbbA gene and all the B. fargilis strains carried the cepA gene. Of the 15 amoxicillin/clavulanate-resistant (fixed ratio) B. fragilis strains 12 carried the cfxA gene while from the 13 amoxicillin/clavulanate-susceptible (fixed ratio) B. fragilis strains none ($p<0.00001$). For the non-fragilis Bacteroides strains included in the study (10 resistant and 12 susceptible to amoxicillin/clavulanate-resistant at a fixed ratio) all but one were cfxA gene positive. In the cfxA-positive B. fragilis strains the Tyr227 was usually variant, however, in the non-fragilis Bacteroides strains the Tyr227 dominated. In the cases of the upstream regions of the cfxA genes we found an opposite relation – for B. fragilis it was mostly uniform (1.2 kb), but for the non-fragilis Bacteroides for the strains susceptible to amoxicillin/clavulanate and piperacillin/tazobactam it was 1.2 kb, contrary to the resistant ones where it was mutated. The β-lactam/β-lactamase combination resistance was transferable in conjugation experiments for one studied strain out of five.

Conclusions: As summary, we can say that mainly the cfxA genes are responsible for β-lactam/β-lactamase inhibitor combination resistance in Bacteroides and B. fragilis and non-fragilis Bacteroides strains differ in the possible manifestation - amino acid change or activation mechanism.

Environmental Microbiology and Biotechnology , -

Investigation of Vibrio cholerea from Hungarian natural bathing waters

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The name of Vibrio cholerae invokes the thought of cholera, the waterborne disease characterized by diarrhea, vomiting, convulsions and dehydration. The symptoms are primarily

caused by the cholera toxin produced by the bacteria. However, there are non toxin-producing V. cholerae bacteria (NTVC or non-O1/O139 Vibrio cholerae, NOVC) which do not induce classical cholera disease, but can cause other intestinal and extraintestinal symptoms. The most common conditions related to NTVC are wound infection and ear inflammation, but soft tissue infection or sepsis are also possible

Non-toxinogenic V. cholerae bacteria are also associated with aquatic environments, and are primarily found in warmer (> 15°C) natural waters with a higher pH and salt concentration.

In recent years, National Center for Public Health and Pharmacy (NCPHP) has identified non-toxinogenic V. cholerae in several clinical samples. In the most cases the epidemiological investigation linked infections to bathing water exposure. The presence of NTVC was verified by environmental epidemiological investigations in the corresponding pools and natural bathing waters.

As a result of climate change, the temperature of domestic natural bathing waters is rising, creating an optimal environment for the proliferation of Vibrio bacteria. The increasing number of human diseases justified the assessment of Vibrio cholerae prevalence in natural bathing waters. To this end, local public health authorities collected samples in all non-flowing water bodies used for bathing as part of their yearly workplan.

. Vibrios were detected by membrane-filtration and culture on TCBS agar. Typical colonies were identified by MALDI-TOF-MS analysis. Isolates belonging to the genus Vibrio, the presence or absence of the cholera toxin gene was investigated by real time PCR.

95% of the the 61 monitored bathing waters contained V. cholerae, counts were between 1 and 40,000 colony forming unit/100 ml and all tested isolates were NTVC. We thank the government offices and the public health laboratories for their work.

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Molecular and Clinical Microbiology , -

Enhanced antibacterial effects of liposomal encapsulated antibiotics against Chlamydia trachomatis serovar D

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Chlamydia trachomatis (C. trachomatis) is a Gram-negative, coccoid eubacterium. There are more known serovars which are related to more serious diseases. Trachoma caused by serovars A-C can cause blindness whereas other serovars can cause sexual transmitted diseases (STDs) (D-K: Non-gonococcal urethritis; L1-L3: lymphogranuloma venerum). C. trachomatis has become a global public health challenge and therefore enhanced efforts are needed to improve our knowledge and to find new therapic oppurtunites. Ciprofloxacin has shown to be effective against C.trachomatis. Trimethoprim-sulfamethoxazole (TMP-SMX) is commonly used to treat STDs, but it has never been used in in vitro studies. Usage of liposomes has several advantages: a) We can reduce doses of antibiotics since odds of drugs deactivation also reduces. b) Liposomes can fuse with target cell membrane and thus accidental loss of drugs can be avoided. c) Nevertheless, we can also reduce the side effects. In this study, we investigated whether liposomal encapsulation can improve the antichlamydial effect of antibiotics. We tested the antibacterial acticity in HeLa 229 cells infected with C.trachomatis serovar D. SigmaAldrich Liposome Kit was used for encapsulation. Real time

quantitative polymerase chain reaction (RT- qPCR) method was used to determinate chlamydial genome content 48 hours after infection. While the encapsulated ciprofloxacin has shown a similar efficyacy, Minimal Inhibition Concentration (MIC) of TMP-SMX was 16-fold lower than those achieved with the free TMP-SMX. Lyophilized powder based liposome kits have become ineffective within four days that highlight that our knowledge needs to be improved to renew our toolbox in this area.

Molecular and Clinical Microbiology , -

Drug repurposing strategy in bacterial infections: phenothiazine antipsychotics as efflux pump inhibitors

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Background

There are numerous antibiotic resistance mechanisms such as restricted penetration, drug efflux, target modification, destruction/modification of the antibiotics, target switching, and target sequestration. Out of these mechanisms, the overexpression of efflux pumps can result in higher virulence of bacteria and these pumps can contribute to bacterial communication (quorum sensing) and biofilm formation.

Targeting efflux pumps may be an attractive strategy to treat bacterial infections. However, the development and discovery of a new drug require a long timeline and may come with high costs. A potential alternative to reduce the time and costs of drug development is to repurpose already existing drugs for new therapeutic indications. Numerous studies have highlighted that phenothiazine-type antipsychotics possess anti-inflammatory, antimicrobial, anticancer properties, and they may be attractive candidates to combat bacterial multidrug resistance.

Materials and Methods

The efflux pump inhibitory (EPI) activity of phenothiazines was assessed by real-time fluorimetry on Staphylococcus aureus, Escherichia coli, Mycobacterium smegmatis, and Mycobacterium avium strains. The influence of phenothiazine treatment on the relative expression of bacterial pump genes was determined by RT-qPCR. Furthermore, the combined activity of phenothiazines and antibiotics was also investigated on Gram-positive and Gram-negative bacterial strains. Their anti-biofilm activity was assessed using crystal violet staining.

Results

Many of the phenothiazines possess wide-ranging antibacterial activity against Mycobacteria, some Gram-positive and Gram-negative bacteria. The application of a subinhibitory concentration of a non-antibiotic substance such as phenothiazines can enhance the susceptibility of bacteria towards antibiotics indicating that efflux mechanisms are involved. Phenothiazines have EPI properties and can regulate the expression of efflux pump and stress genes, depending on environmental factors. Furthermore, they can reduce biofilm formation and can inhibit bacterial communication contributing to decreased bacterial virulence.

Discussion

Phenothiazine antipsychotics have demonstrated efflux pump inhibiting activity that may be utilized in the treatment of bacterial infections alone or in combination with antibiotics. Phenothiazines can reduce the virulence of bacteria and enhance the activity of conventional antibiotics. However, phenothiazines may have direct consequences for the composition and stability of the gut microbiome and may have a synergistic interaction with other drugs provoking dysbiosis.

Molecular and Clinical Microbiology , -

Pathogenic Escherichia coli strains and lytic phages active on enteric bacteria isolated from Hungarian domestic animals

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Foodborne pathogenic enterobacteria in domestic animals pose a serious health risk and food safety problem. Because of the emerging antibiotic resistance among these bacteria, their biocontrol using bacteriophages is an increasingly studied potential solution. In the current study, we aimed to isolate and characterise pathogenic enterobacteria and lytic bacteriophages from enteric samples of domestic animals collected throughout Hungary. A total of 161 samples of animal origin (bovine and porcine, as well as samples from lamb, rabbit, chicken, pheasant, ostrich, and pigeon) were taken. In addition, 81 bovine E. coli strains of earlier isolation were received for characterisation. Shiga toxigenic (STEC) strains were isolated from five bovine samples (20 isolates), of which four contained enterohemorrhagic (EHEC, 17 out of the 20 isolates) strains. Enteropathogenic strains (EPEC) were isolated from two bovine and two rabbit samples (14 isolates), and enterotoxigenic (ETEC) strains from two porcine, six bovine, and six chicken samples (69 isolates). Three bovine samples yielded STEC-ETEC hybrid strains (nine isolates), which is the first instance of such strains being isolated in Hungary. Of the 81 bovine strains, there were 13 STEC (one of them EHEC), 2 EPEC, and 6 necrotoxigenic (NTEC) strains. Phage induction was attempted on the STEC strains using mytomicin-C, norfloxacin, and UV light. Prophages were induced from nine STEC strains, which included six of the nine STEC-ETEC hybrid strains. Lytic bacteriophages, as well as their enzymes and other protein components, are increasingly coming into the focus of research on biocontrol, as well as novel and alternative antibacterial agents. From the same animal sample set, we isolated 140 stocks of lytic bacteriophages, detecting them by activity shown on E. coli K-12 (n=72), EHEC O157:H7 Sakai (n=12), Citrobacter rodentium (n=43), Yersinia enterocolitica (n=6), and Y. pseudotuberculosis (n=8) strains. The detailed phenotypic and genotypic characterisation of these phages could reveal new taxonomic groups of phages and those with applicability for antibacterial purposes. This study was supported by the National Research, Development, and Innovation Office (grant no. NKFI FK 143174).

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Evaluation of the potential of selenoesters to reverse multidrug resistance

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Background

Multidrug resistance (MDR) poses a significant challenge in treating bacterial infections and cancer. MDR refers to the ability of microorganisms or tumor cells to resist multiple drugs, making conventional therapies ineffective. Antimicrobial resistance mechanisms include reduced drug uptake, target modification, drug inactivation, and active drug efflux. Overexpression of efflux pumps can also increase bacterial virulence and affect biofilm formation. In cancer, drug resistance is driven by mechanisms such as increased drug efflux, genetic factors, avoiding apoptosis, enhanced DNA repair. These factors reduce the effectiveness of cancer treatments, making tumor management more difficult. Recently, interest has grown in selenium (Se), Se-nanoparticles, and selenocompounds particularly against multidrug resistant bacteria and tumor cells. Se-compounds have unique properties acting as both antioxidants and prooxidants. As antioxidants, Se-compounds like selenocysteine help maintain redox balance and protect cells from oxidative stress. As prooxidants, they can induce significant ROS production, leading to oxidative stress within cells.

Materials and methods

The minimum inhibitory concentrations (MICs) of the selenoesters were evaluated against both Gram-positive sensitive and resistant bacterial strains, as well as Gram-negative strains. The disruption of mature biofilms and anti-biofilm activity were assessed using biofilms formed by Pseudomonas aeruginosa (CCM 3955) and Staphylococcus aureus (ATCC 25923). To evaluate anti-efflux pump activity, Salmonella Typhimurium and S. aureus strains were applied. Regarding anticancer activity, Se-compounds were investigated on doxorubicin-sensitive human colonic adenocarcinoma cell line (Colo 205; ATCC-CCL-222), the multidrug resistant P-gp expressing (MDR1)-LRP human colonic adenocarcinoma cell line (Colo 320; ATCC-CCL-220.1) and normal, human embryonal lung fibroblast cell line (MRC-5; ATCC CCL-171). The P-gp inhibitory effect of the compound was tested using the rhodamine 123 assay. Furthermore, the apoptosis-inducing effect of selenoesters was investigated by annexin V-FITC/propidium iodide staining.

Results

Assessing the antibacterial effects, the ketone-selenoesters proved to be more effective than the cyano-selenoesters. All of the Se-compounds exhibited anti-biofilm properties, and efflux pump inhibition was demonstrated in S. aureus MRSA and Salmonella Typhimurium strains. Cyano-selenoesters were highly toxic on colon cancer cell lines but had no impact on normal MRC-5 cells. According to the results obtained by flow cytometry, some of the ketone-selenoesters exhibited potent P-gp inhibition and induced apoptosis in Colo 320 cells.

Conclusion

Selenium-containing compounds could offer alternative and effective solutions for combating MDR in bacteria and tumor cells. However, further investigation is needed to fully understand the mode of action of these

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Carnosic acid inhibits herpes simplex virus replication by suppressing cellular ATP synthesis

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To identify novel antiviral compounds, the antiviral activity of eight plants indigenous to the Southern region of Hungary against herpes simplex virus-2 (HSV-2) was investigated.

The plant extracts and the plant compound carnosic acid were tested for their effectiveness on both the extracellular and/ or the intracellular forms of HSV-2 on Vero and HeLa cells. HSV-2 replication was measured by a direct quantitative PCR.

Among the plant extracts tested, Salvia rosmarinus (S. rosmarinus) exhibited a 90.46% reduction of HSV-2 replication at the 0.47 μg/ml concentration. Carnosic acid, a major antimicrobial compound found in rosemary also demonstrated a significant dose-dependent inhibition of both extracellular and intracellular forms of HSV-2. The 90% inhibitory concentrations (IC90) of carnosic acid were between 25-6.25 μg/ml. Proteomics and high-resolution respirometry showed that carnosic acid suppressed key ATP generation pathways such as glycolysis, citrate cycle and oxidative phosphorylation. Inhibition of oxidative phosphorylation also suppressed HSV-2 replication up to 39.94 fold.

Carnosic acid significantly restricted HSV-2 growth in human cervical epithelial cells. The antiviral action could be explained by the inhibition of ATP generation by suppressing key energy production pathways. Carnosic acid holds promise as a potential novel antiviral agent against HSV-2.

Molecular and Clinical Microbiology , -

Serotype distribution and antibiotic susceptibility of carried pneumococci before the introduction of new generation conjugate vaccines PCV15 and PCV20

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In Hungary, the first pneumococcal conjugate vaccine (PCV-7) has been officially recommended since 2009, and PCV-13 was made obligatory in 2014. Pneumococci are frequent colonisers of the human nasopharynx, especially in young children, who represent the major reservoirs for infections. The conjugate vaccines place a huge selection pressure on this species, leading to quite radical serotype re-arrangements. As two new-generation conjugate vaccines (PCV-15 and PCV-20) are now available, our aim was to survey the current epidemiological situation by preparing a snapshot before vaccine introduction.

401 healthy children attending communities, aged 1-7 years, were screened between April 2022 and April 2023. Samples from both nostrils were collected and inoculated on blood agar plates. Pneumococcal colonies were identified, subcultured and confirmed by optochin sensitivity and PCR detection of the lytA gene. The serotype of the isolates was determined by the Pneumotest Latex Kit and fine typing was made by PCR. The antibiotic susceptibility was primarily determined by the VITEK 2 AST-P576 cards (bioMérieux) to 17 drugs, and resistant strains were additionally tested with a gradient MIC test strip.

The carriage rate was 16.5% (n=66), which is much lower compared to previous observations. The six most prevalent serotypes in ranking order were 23B, 35F, 15A/F, 15B/C, 11A and 23A. The coverage of PCV13, PCV15 and PCV20 would be 9.1%, 10.6% and 31.8%, respectively, over this strain collection. None of the isolates were resistant to penicillin, but 16 strains fell into the intermediate category. Resistance was found to macrolides (n=8), tetracycline (n=7) and TMP/SMX (n=16). Only three strains were multiresistant (i.e., to three different antibiotic classes). Serotypes 15A, 23B and 19F contributed mostly to resistance. Although the gender of the tested children was very much equalised, male dominance was observed among the carriers (50.6% versus 60.6%). The other major risk factor was having otitis media in the past, whereas passive smoking had a negatively association with carriage.

In conclusion, conjugate vaccines not only suppress the vaccine serotypes, but they can reduce pneumococcal carriage in general. Hence, they have a key role in disease prevention, also in the elderly, through herd effect. At the moment, PCV13 coverage is very low, but that of PCV20 is still significant (31.8%). Fortunately, the replacing non-vaccine serotypes are not associated with high resistance. However, continuous monitoring of serotype re-arrangement is necessary, especially now, at the gate of PCV20-induced changes.

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Molecular and Clinical Microbiology , -

Introduction of qPCR-based diagnostics in routine laboratory for bloodstream infections

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One of the most important samples in microbiological laboratories is hemoculture. The gold standard method of diagnosing bloodstream infections is using a haemoculture bottle. Incubation is automated, the detection of a positive signal can take from a few hours to days. Readings of direct resistance after microscopic examination of positive bottles can be taken the next day. This minimum of 2-3 days means a long time in patient care, accelerating this process is of primary importance for effective antibiotic treatment of the patient.

Among the multiplex diagnostics available on the market, we chose Alifax's Molecular Mouse multiplex qPCR based system, primarily because of its affordable price. The system detects 44 microorganisms and 20 resistance genes distributed in five panels. We had the opportunity to try four of the five possible panels, one identification and one resistance, and two combined (identification and resistance) panels. Nearly a hundred samples were examined, of which 30% were Gram-negative and 70% Gram-positive pathogen detection. The presentation details the measurement experiences and results.

To summarize the experiences, we can conclude that due to the panel structure, it is primarily optimal for the detection of a single pathogen. The greatest benefit of the system is seen in the rapid diagnosis of severe Gram-positive bloodstream infections, especially in the detection of infections caused by *Streptococcus pyogenes*. Although the assembly of the panels was made for the international market, so they do not fully cover the pathogenic spectrum occurring in bloodstream infections in Hungary, the manufacturer is open to modifying the panels in case of serious demand. The feedback from clinicians is very positive, rapid species determination and prediction of possible resistance genes greatly help the application of appropriate therapy. In possession of the direct resistance results, there was little need to modify the therapy recommended based on the qPCR partial result.

Molecular and Clinical Microbiology , -

A comparative study of human Saccharomyces isolates in a Hungarian clinical center: phylogenomics, clades, and antimycotic susceptibility

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A lesser-known aspect of the ubiquitously used yeast Saccharomyces cerevisiae is that it may colonize and even infect the human body under certain circumstances. The pathogenicity and diversity of clinical isolates of this species are not yet fully understood. S. cerevisiae var. 'boulardii' is a probiotic yeast recognized as a subtype of S. cerevisiae. It is primarily utilized for the prevention and treatment of diarrhea. Like other members of the genus, S. ‘boulardii’ is generally considered as a safe microorganism. However, both S. cerevisiae and its probiotic subtype have been reported to cause fungemia in vulnerable populations, such as infants, the

elderly, and immunocompromised patients, highlighting the need for focused investigation on the pathogenic potential and epidemiology of these yeasts.

In our current study, Hungarian clinical isolates of S. cerevisiae were collected for comparative analysis. These samples were obtained from the Clinical Center of the University of Debrecen over an eight-year period, during which more than 50 clinical isolates were examined. No increasing trend in the prevalence of samples was observed throughout the timeframe. Regarding patient demographics, nearly half of the isolates were from elderly patients, about one-fifth from children, and 60% of the samples were from female patients. Four cases involved mycosis diagnoses: three were derived from blood cultures, and one was isolated from a cervical sample. In terms of origin, 60%, 25%, and 5% of the samples were obtained from the respiratory tract, female genital tract, and bloodstream, respectively.

We employed multiplex PCR fingerprinting to identify samples genetically classified in probiotic subtype. Our findings indicated that approximately 40% of the isolates were of probiotic origin. The age distribution of these samples was consistent with previous literature, where most isolates were from children and elderly patients. Interestingly, the majority of clinical isolates were associated with the respiratory tract, a trend so far not reported for the species.

These results suggest that S. ‘boulardii’ may exhibit opportunistic pathogenicity. The probiotic yeast can lead to fungemia, particularly in catheterized, immunocompromised patients with prolonged hospital stays, highlighting the significance of its biofilm-forming capability in clinical practice. We also evaluated the susceptibility profiles of the isolates in planktonic forms to five antifungal agents. Notably, the susceptibility of the probiotic subtypes to amphotericin B showed significant differences. The observed minimum inhibitory concentrations were often high when compared to typical opportunistic pathogenic yeasts.

In conclusion, S. cerevisiae emerged as a rare opportunistic pathogen or colonizer at the Clinical Center of the University of Debrecen, with a substantial proportion of isolates exhibiting molecular characteristics of the probiotic clade.

Molecular and Clinical Microbiology , -

Epidemiological characterisation of Pneumocystis jirovecii pneumonia diagnosed at the University of Debrecen, Clinical Centre

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Pneumocystis jirovecii is an opportunistic fungal species, which is associated with even life-threatening pneumonia especially in case of immuncompromised patients. Predisposing factors for this disease include impaired cell-mediated immunity caused by HIV infection, several inflammatory and rheumatologic conditions as well as solid organ transplantation. Currently, the definitive diagnosis from lower respiratory secretions or tissue is challenging because clinical symptoms are usually non-specific. Current study evaluated the clinical characteristic of P. jirovecii pneumonia and reveal the diagnostic possibilities related to this opportunistic pathogen (ethical permission number: 5775-2021). A total of 97 requests towards Pneumocystis from the University of Debrecen, Clinical Centre, between January 2021 and July 2024 were investigated retrospectively. We evaluated the clinical characteristics of cases including comorbidities, predisposing factors, mortality and applied diagnostic algorithm of positive cases. Regarding the observational period, 13 out of 97 cases showed definitive P. jirovecii infection. The highest positivity rate was detected at 2023 (9 cases). Haematological

malignancies including acute lymphoblastic leukemia, peripheral T cell lymphoma, Hodgkin's lymphoma and Non-Hodgkin's lymphoma were detected at 7 out of 13 cases. The 30-day mortality rate was 46%. It is noteworthy, that all fatal outcomes were associated with intensive care units. Concerning our diagnostic algorithm, both PCR-based diagnosis and serological investigations (beta-d-glucan) were performed, where the measuring serum beta-d-glucan is a useful tool for supporting a quantitative PCR-based diagnosis of suspected Pneumocystis with bronchoalveolar lavage fluid. Our comprehensive analysis supports the better characterisation of P. jirovecii pneumonia in healthcare-settings; furthermore, it provides valuable data to limited Hungarian epidemiological characteristics.

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Molecular and Clinical Microbiology , -

Epidemiological properties of bloodstream infections caused by Candida species at University of Debrecen, Clinical Centre

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Large-scale epidemiological studies showed that the overall pooled-incidence of Candida bloodstream infections is 3.88/100,000 in European countries, from which the highest number was observed in intensive care units (5.5/1000 admissions). The proportion of Candida albicans-related episodes have declined significantly from 70%–80% to 40%–60%, depending on given countries. However, the ratio of non-albicans-associated candidaemia has increased significantly. In curren study, a total of 149 candidaemia episodes from the University of Debrecen, Clinical Centre, between January 2020 and December 2023 were involved retrospectively. The susceptibility profile of Candida isolates to fluconazole, amphotericin B, anidulafungin, caspofungin, and micafungin was tested and compared to the susceptibility of 1-day-old biofilms developed by Candida isolates. C. albicans was the most frequently isolated species (41%), followed by C. parapsilosis (20%), C. glabrata (14%), C. tropicalis (13%), and other so-called rare species (7%) including Cyberlindnera fabianii, C. lusitaniae, C. guilliermondii and C. metapsilosis. One episode of C. dubliniensis was also detected. The lowest prevalence was observed in the case of C. krusei (5%). Overall, the 30-day mortality rate was 52%. Regarding the applied antifungal agents, fluconazole was the most frequently used antifungal drug (53%), followed by anidulafungin (15%), caspofungin (10%), and micafungin (6%). In the multivariate regression analysis, we examined the factors influencing 30-day mortality in patients with candidaemia. Based on our results, intensive care unit admission (odds ratio [OR]: 2.99; 95%CI: 1.17–8.04; $p = .025$) and fluconazole therapy (OR: 4.12; 95%CI: 1.62–11.42; $p = .004$) were independent predictors of mortality. Our comprehensive analysis supports the better characterisation of candidaemia in healthcare settings, which ultimately may reduce mortality among patients.

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Molecular and Clinical Microbiology , -

Occurrence of Toxin-Producing Clostridioides difficile Ribotypes Predictable by MALDI-TOF Mass Spectrometry in the Southern Great Plain Region, Hungary

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Introduction: The rapid and accurate ribotyping of Clostridioides difficile infections is crucial for developing effective infection control strategies. Today, this can be achieved through further analysis of MALDI-TOF MS data.

Materials and Methods: In our study, we analyzed the mass spectra of 145 C. difficile strains isolated in our institute in 2024 using the machine learning algorithms available on the website https://bacteria.id/. The website automatically predicted whether the strains belonged to either of the two most significant hypervirulent ribotypes, RT027 and RT181, or other ribotypes. These data were compared with the in vivo and in vitro toxin production capabilities of the strains.

Results: Of the 145 strains examined, 67 were identified as RT181 ribotype, while only 11 belonged to the RT027 ribotype. Almost all strains classified into the two ribotypes were found to produce toxins under in vivo or in vitro conditions. In 63 strains, toxin production could only be confirmed retrospectively using the in vitro "toxigenic culture" technique, out of which 23 were identified as RT181 ribotype. Of the strains predicted to be either RT181 or RT027 ribotype, only one RT027 strain was toxin-negative (1.27%). All RT181 strains were toxin producers.

Conclusion: The freely accessible https://bacteria.id/ website was able to predict the toxin production of C. difficile strains from MALDI-TOF mass spectra with a 98.73% success rate. This new methodology significantly reduces the time and cost of typing while providing a valuable tool for researchers and infection control professionals for the rapid determination of ribotypes.

Molecular and Clinical Microbiology , -

Impact of the COVID-19 Pandemic on the Prevalence and Antibiotic Resistance of Corynebacterium striatum in a Hungarian Clinical Center

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The role of Corynebacterium striatum in various nosocomial infections has been increasingly recognized. During the COVID-19 pandemic, its virulence, particularly in the respiratory tract of immunosuppressed patients, has garnered significant attention. This retrospective study, conducted at the Clinical Center of the University of Szeged, Hungary, from 2012 to 2021,

aimed to investigate the prevalence and antimicrobial resistance patterns of C. striatum. A total of 498 positive samples from 312 patients were included, with 4529 antibiotic susceptibility tests performed on the isolates. The findings revealed a significant increase in the prevalence of C. striatum during the pandemic, especially in respiratory, blood culture, and superficial samples. Resistance to rifampicin and linezolid notably increased during the study period, likely independent of the commonly isolated co-pathogens.

A second study focused on the rise of rifampicin resistance in C. striatum during the same period. Data collected from January 1, 2012, to December 31, 2021, at the Department of Medical Microbiology, University of Szeged, were analyzed to characterize resistance trends using the antibiotic resistance index (ARI). Fourier-transform infrared spectroscopy (FTIR) with the IR Biotyper® was employed to type 14 strains with different resistance patterns. The study suggests that the increased rifampicin resistance observed during the COVID-19 pandemic may be associated with the use of Rifadin® for treating co-infections with Staphylococcus aureus. FTIR analysis indicated that the rifampicin-resistant C. striatum strains were closely related, supporting this hypothesis.

Overall, the data indicate that the COVID-19 pandemic has significantly impacted the incidence and antimicrobial resistance patterns of C. striatum at the University of Szeged. The increasing resistance to rifampicin and linezolid, coupled with the diverse and evolving nature of co-pathogens, underscores the necessity for vigilant antimicrobial stewardship. The study predicts that vancomycin may remain the only effective agent by 2030 if current trends continue.

Molecular and Clinical Microbiology , -

Development of molecular assays for the detection of antibiotic susceptibility in Mycoplasma iowae

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Mycoplasma iowae is an economically important pathogen in turkeys, which causes a reduction in hatchability, late embryo mortality and leg deformities, chondrodystrophy and skeletal lesions in poults. At present, no vaccine is available for this bacterium. Therefore, while prevention is mainly relied on adequate housing and biosecurity measurements, the most effective method for reducing economic losses during an outbreak is the administration of an appropriate antibiotic for the targeted treatment of the infection.

The objective of the study was to identify single nucleotide polymorphisms (SNPs) associated with elevated antibiotic minimum inhibitory concentrations (MICs) in 99 M. iowae strains and to develop molecular biological assays to detect the resistance markers.

The whole genome sequences of 99 M. iowae strains were determined with Oxford Nanopore and/or Illumina sequencing platforms, while the MIC values were gained by the broth micro-dilution method. A bimodal distribution of MIC values was observed for fluoroquinolones, macrolides and lincosamides, which enabled further analyses in search of point mutations showing correlations with elevated MIC values. Mismatch amplification mutation assays (MAMAs) were designed for the differentiation of the point mutations in the corresponding regions.

SNPs associated with antibiotic resistance were identified in the gyrA, parC and 23S rRNA genes. SNPs were identified in the gyrA gene at nucleotide position 279, and in the parC gene at nucleotide position 2021 (Escherichia coli numbering). Although a resistance-associated mutation at nucleotide position 2059 in the 23S rRNA gene was also identified, the presence of all four nucleotides at this position represents a challenge for the development of a molecular detection system. The MAMA assays clearly distinguished the genotypes corresponding to low or high MIC values, showed 103 template copy number/µl sensitivity and exhibited no cross reactions with other bacteria.

The developed MAMAs represent time-efficient and cost-effective methods that can also be used on clinical samples to identify M. iowae strains with elevated MIC values for fluoroquinolones. Accordingly, the use of the designed molecular tools could help in pathogen control and targeted antibiotic therapy during outbreaks.

Molecular and Clinical Microbiology , -

The Occurrence of *Chlamydia felis* in Cats and Dogs in Hungary

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The World Health Organization (WHO) estimates that many human infections are zoonoses, creating a worldwide public health challenge. Among *Chlamydia* species, *Chlamydia felis* is the leading cause of conjunctivitis in cats and is a prominent zoonotic species. This study aimed to determine the occurrence and risk of chlamydiosis in cats and dogs in Szeged, Hungary, and surrounding areas. The total nucleic acids from conjunctival swab samples of symptomatic and asymptomatic animals were extracted using an automated nucleic acid extraction system. After that, DNA was amplified by pan-chlamydia PCR. Bacterial and fungal cultures were also performed to detect other microorganisms. Of the 93 animals, 32 (34.4%) were positive for pan-chlamydia PCR. The positivity rates were 33.3% (26/78) in cats and 40.0% (6/15) in dogs. Furthermore, the positivity rates were 37.2% (16/43) in the cat shelter, 42.4% (14/33) in the veterinary clinic, and 11.7% (2/17) in household pets. In total, 103 species were identified through culture-based examinations, including 97 (94.2%) bacterial and 6 fungal (5.8%) species. From both human and animal health perspectives, it is essential to have a detailed understanding of the circumstances of chlamydiosis, given the global impact of zoonotic diseases.

Environmental Microbiology and Biotechnology, -

Development of efficient expression system for ochratoxin A detoxification

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Filamentous fungi that colonize food and feed crops, such as species within the Aspergillus and Penicillium genera, can produce ochratoxin A (OTA) in response to environmental stress. OTA represents a significant health hazard for both humans and animals due to its nephrotoxic,

immunosuppressive, and potentially carcinogenic properties, which contribute to the damage of various organ systems, including the kidneys, liver, and nervous system. Our research focuses on the Cupriavidus basilensis ŐR16 bacterial strain, which demonstrates approximately 100% efficacy in the biodegradation of OTA within 5 days. This strain possesses several enzyme candidates capable of OTA degradation, including an amidohydrolase identified in the literature as being active against OTA. This amidohydrolase catalyses the hydrolysis of OTA, leading to the detoxification of the mycotoxin into ochratoxin α and phenylalanine. We investigated the expression efficiency of the gene encoding this amidohydrolase and its ability to degrade OTA using both homologous – Cupriavidus plantarum LMG 26296, and heterologous – E. coli BL21(DE3) expression systems. The degradation potential of the transformants and the metabolites produced, and their toxicity were verified by HPLC and cytotoxicity assays. Our results provide evidence for the detoxification efficiency of amidohydrolase and, using homologous and heterologous expressed transformants, we highlight the differences in enzyme activity in different hosts and the optimal expression conditions required for efficient degradation of OTA.

Keywords: mycotoxin, ochratoxin A, enzymes, protein expression system

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Environmental Microbiology and Biotechnology, -

Relevance of Patescibacteria in a hydrocarbon contaminated groundwater

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Patescibacteria, formerly known as a member of the Candidate Phyla Radiation (CPR) and recently proposed phylum of bacteria have been identified as one of the most enigmatic bacterial group due to its notable diversity and distribution across various environments, their unusual genetic makeup and relatively small cell size. Members of Patescibacteria phylum have been identified in a wide range of environments like soil, sediment, marine environments and even within the human gut.

Our investigation focused on a peculiar environment, we analyzed the diversity and distribution of Patescibacteria in a hydrocarbon contaminated aquafer. First and foremost, we studied the diversity of Patescibacteria with commonly used primer pair targeting the V3-V4 region of the 16S rRNA gene. Additionally, we tested a novel Patescibacteria-specific 16S rRNA gene amplicon sequencing primer pair.

Unsurprisingly, the most dominant phylum was Proteobacteria in all four samples, followed by Bacteroidetes, Firmicutes and Patescibacteria. Applying the novel Patescibacteria-specific primer pair we aimed to shed light on the real composition of the community since the abundance and diversity of Patescibacteria is frequently underestimated. According to our results, we could state that novel Patescibacteria-specific primer pair is not ultimately increase the abundance of Patescibacteria, only in cases where we could formerly observe a higher

abundance. Additionally, on genus level this primer pair increased not only the abundance of Patescibacteria genera as Ca. Shapirobacteria, Ca. Woesebacteria, Ca. Kaiserbacteria and Saccharimonadaceae but also the diversity, too.

Keywords: Patescibacteria, 16S rRNA gene amplicon sequencing, BTEX-contaminated groundwater;

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Environmental Microbiology and Biotechnology, -

Feedstock-driven antibiotic resistance gene dynamics in industrial biogas plants

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This study investigated antimicrobial resistance in the anaerobic digesters (AD) of two industrial-scale biogas plants processing agricultural biomass and municipal wastewater sludge. A combination of deep sequencing and machine learning-guided genome-centric workflow was implemented for metagenomic and metatranscriptomics data analysis to comprehensively examine potential antimicrobial resistance in microbial communities. AD microbes were found to harbour numerous antibiotic resistance genes (ARGs), with 58.85% of the metagenome-assembled genomes (MAGs) harbouring antibiotic resistance. A moderately positive correlation was observed between the abundance and expression of ARGs. ARGs were located primarily on bacterial chromosomes. A higher expression of resistance genes was observed on plasmids than on chromosomes. Risk index assessment suggests that most ARGs identified posed a significant risk to human health. However, the potentially pathogenic bacteria showed lower ARG expression than non-pathogens. Resistomes at the gene category level were found involved in multidrug resistance and resistance to beta-lactam, glycopeptide, peptide and macrolide-lincosamide-streptogramin resistance. Differential expression analysis revealed specific genes associated with potential pathogenicity, emphasizing the importance of active gene expression in assessing the risks associated with ARGs. While anaerobic treatment clearly reduced the activity of potentially pathogenic microbes, the efficiency of this approach is limited.

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Environmental Microbiology and Biotechnology, -

Climate change-induced taxonomic diversity fluctuations of extremophilic bacteria from a high-altitude meltwater pond on Ojos del Salado (Chile)

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Permafrost is strongly affected by climate change, thus studying the responses and adaptations of extremophilic bacterial communities to rapid changes is key to advancing our knowledge of extreme environments that remain free from anthropogenic influences. This study focused on a high-altitude seasonal meltwater pond, a multiple extreme habitat, located in the Altiplano Plateau at 5900 m on the Ojos del Salado (Dry-Andes, Chile). Sampling of permafrost, pond sediment, and water samples was taken during three subsequent expedition years with distinct weather conditions (average, high precipitation, severe drought). Novel cultivation combinations uncovered the hitherto little-known polyextremophilic bacteria, identified as Actinobacteria, Proteobacteria, and Firmicutes representatives. Illumina MiSeq amplicon sequencing was employed following environmental DNA extraction. The 16S rRNA gene-based sequencing results indicate that the relative abundance of dominant taxa (Proteobacteria, Bacteroidota, Acidobacteriota, and Verrucomicrobiota) exhibited high variability in response to sample types and weather conditions. Strong moisture-dependent quantitative changes were detected in the case of phyla Cyanobacteria and Actinobacteriota. Predominant sequence reads were linked to orders Chitinophagales, Burkholderiales, Flavobacteriales, and Sphingomonadales, recognized for their proficiency in degrading various organic compounds. This research was supported by the National Research, Development and Innovation Office, Hungary (Grants NKFIH OTKA K147424).

Environmental Microbiology and Biotechnology, -

Characterisation of fungal communities in the roots of orchid species of the genera *Epipactis* and *Cephalanthera* in native and alien poplar monocultures

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The environmental value of poplar monocultures is generally low and their conservation role is often overlooked. However, recent research shows that these plantations can provide important habitat for several early-successional orchid species, including some endangered endemic species. In this presentation, we report for the first time data on the composition of fungal communities in poplar monoculture soils and in the roots of *Epipactis* and *Cephalanthera* orchids using environmental DNA-based methods. A total of 1572 fungal genotypes were identified and classified into functional groups in 58 root and soil samples analysed. The sampled communities were dominated by ectomycorrhizal fungi (299 genotypes), generalist saprotrophs (265), and plant pathogens (135). Overall, the composition

of the fungal communities in the soil and root samples differed significantly, but several ectomycorrhizal fungi were found in both soil and orchid roots. This supports the partial mycoheterotrophic lifestyle of these orchids, which provides indirect access to the roots of poplar trees as a carbon source for the orchid through symbiotic fungi shared with poplar trees. While species of the phylogenetic lineages /cortinarius, /geopora, /hebeloma-alnicola, /inocybe and /russula-lactarius were mainly found in soil samples, various species of the clades /tomentella-thelephora and /tuber-helvella dominated in orchid roots, of which truffle (*Tuber*) species were particularly enriched in roots relative to soil. Differences were also found between the two orchid genera in terms of the richness, relative abundance and composition of their fungal symbionts: the roots of *Epipactis* were dominated by *Tuber* species and the roots of *Cephalanthera* by *Tomentella* species. Of the environmental variables measured, soil chemistry and potassium content showed the greatest effect on the composition of both the total and ectomycorrhizal fungal communities. Finally, several fungal indicator species were identified in poplar plantations with and without orchids.

Environmental Microbiology and Biotechnology, -

Bacterial communities developing in the rhizosphere of Szarvasi-1 energy grass on dredged mud from lake Balaton

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The aim of the present research is to reveal the prokaryotic community structures from the risoshphere of Szarvasi-1 energy grass (Elymus elongatus subsp. ponticus) after 3 months cultivation (in a reclamation project) on dredged mud.

The experiment was conducted outdoors in fifty containers of one m3 volume, filled with dredged mud or garden soil available commercially (used as a control). During the experiments, nine different treatments were applied: mycorrhizal and bacterial inoculants (Bactofil A10 and Soil Basic by AGRO Bio Hungary Kft.) alone and combined with artificial fertilizers (Substral by VOG Export-Import Kft. and Oázis) to observe their effects on plant growth.

To determine the microbial community composition control mud and soil samples (at the beginning and after 3 months) as well as rhizosphere samples (after 3 months) were taken. From the samples community DNA was extracted using the DNeasy PowerSoil Pro Kit (QIAGEN) and amplicon sequencing was carried out (Illumina Myseq). The resulting sequence data were processed using the mothur program (https://mothur.org). The chemical composition of the samples was also determined, and was recorded together with plant growth.

Chemical analysis showed that the mud samples were richer in nutrients, macroelements and trace element contents compared to the control soil. All of the heavy metals were below the regulatory limits.

During the short period of plant growth no significant differences were observed as a result of any treatments, with average shoot lengths ranging from 16.8 to 20.4 cm.

After 3 months cultivation, differences in the prokaryotic community composition from the risoshphere could be observed by environmental factors (e.g. light exposure and connected temperature case of the sun-exposed containers and containers from shaded areas). In the

samples from containers exposed permanently to sun, the members of the Actinobacteriota phylum were dominant, their average relativ abundance was 35.7%, while the Pseudomonadota phylum showed dominance in the samples from „shaded" containers, their average relative abundance was 46.74%. Representatives of the Chloroflexi, Gemmatimonadota, Bacteroidota, and Firmicutes phyla were also present in all samples.

Among the detected taxa several, presumably PGPM bacteria could be identified: Among Alphaproteobacteria members of the family Rhizobiaceae were found in every sample, with a relative abundance exceeding 3%, and members of the Bejerinckiaceae, Azospirillaceae, and Xanthobacteriaceae families were also present. Some of them are known as nitrogene fixing bacteria or siderophore producers.

Environmental Microbiology and Biotechnology, -

Biodeterioration of cement concretes in wastewater treatment facilities: Microbial composition of the concrete biofilms

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Cement biodeterioration in concrete-based wastewater treatment plants (WWTPs) is a serious concern linked to frequent crack formation. This necessitates regular maintenance as it poses risks to environmental pollution due to sewage leakage. It is usually the result of biogeochemical interactions between microbial metabolites and the alkaline mineral phases in hydrated cement leading to the formation of deterioration products.

The present work aims to analyze the microbial communities and their possible roles in cement biodeterioration in sewage gases (in a pumping station) and sewage liquid (in a sandtrap) of WWTP facilities. This study was an in-situ exposure experiment, carried out for different periods:10, 30, 75, 150, and 240 days. It involved 20 cement paste samples in equal numbers of two cement types - ordinary Portland cement (OPC) and calcium sulfoaluminate cement (CSA). From both cement categories, one specimen was kept unexposed in laboratory conditions as a reference for comparison. After each exposure period, deteriorated samples were removed from each site for analysis. Geochemical techniques (SEM and XRD) were used to characterize the chemical and mineralogical composition of the samples. Thereafter, DNA was extracted from the surface biofilms, and after amplicon sequencing (Illumina Miseq) of 16S rRNA genes of Bacteria and Archaea communities of the samples were analyzed, respectively. Bioinformatic analysis was done by Mothur v.1.48.1, whereas statistical analysis was done by using Statigraphics.v18 and Past4.03.

Our results showed that (a) there was a notable change in some physicochemical properties (i.e., size and composition) of the samples. (b) XRD analysis showed the change in the mineral composition of the samples, including the formation of new mineral phases (i.e., ettringite-Ca6Al2 (SO4)3(OH)12·26H2O) and calcite (CaCO3). (c) Microbial analysis showed that the diversity increased with time in case of all samples from both locations. The statistical analysis confirmed that the microbial communities of the samples from the two locations were different. Furthermore, the samples exposed for a longer time (e.g., after 5 and 8 months) clustered independently from those exposed for a shorter time e.g., 1 and 2 months, suggesting the maturation of the biofilms. (d) The microbial communities of the samples from the pumping station were dominated by Proteobacteria (63.5%), followed by Actinobacteriota (21.4%), Firmicutes (5.3%), and Bacteroidota (2.7%), whereas in the sandtrap, the most dominant phylum was Firmicutes (24.98%), followed by Proteobacteria (22.3%), Bacteroidota (12%), Desulfobacterota (10.23%), Actinobacteriota (10%), Campylobacteriota (9.5%), and Synergistota (4.8%). In the biofilm samples, some bacterial taxa were identified which are presumambly responsible for deterioration: sulfide oxidizers (e.g., Thiobacillus, Acidithiobacillus and Halothiobacillus), sulfate reducers (e.g., Desulfomicrobium and Desulfobulbus) and also different fermentative bacteria (e.g. members of Ruminococcaceae, Enterobacteriaceae and Clostridiaceae).

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Proteomic analysis of Rhodococcus erythropolis NI86/21 soil bacterium grown in the presence of aflatoxin B1

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The successful sequencing and annotation of the genome of the soil bacterium Rhodococcus erythropolis NI86/21 allowed us to investigate whether the strain possesses F420H2-dependent reductases that play an active role in the degradation of aflatoxin (AFB1) by Mycolicibacterium smegmatis (1). A BLAST homology searches identified 31 F420H2-dependent reductases in strain NI86/21, but these enzymes showed moderate homology with AFB1 active enzymes of M. smegmatis. The highest homology (69 %) was between the HG717_14625 protein of the NI86/21 strain and the MSMEG_5717 enzyme of M. smegmatis (which had the lowest AFB1 degrading efficiency (specific activity (nmol min-1 mmol-1 enzyme): 3)). However, the enzyme with the highest activity (MSMEG_5998, (specific activity (nmol min-1 mmol-1 enzyme): 83.000)) showed only 51 % identity with HG717_30110. Based on these results, we targeted the mutagenesis of the F420H2-dependent glucose 6-phosphate dehydrogenase (FGD, HG717_20440), which converts the oxidized form of the F420 co-enzyme to a reduced form, and its elimination from the M. smegmatis strain results in the loss of AFB1 degradation. Our attempts in this direction were unsuccessful due to illegitimate recombination events. Our next step was to obtain information on which proteins production is increased in strain NI86/21 in the presence of 1 mg/ml AFB1 concentration in LB medium compared to cells grown only in LB medium.

For this, we chose the unlabelled Lc-MS/MS based protome analysis. A total of 25 proteins were significantly increased in the presence of AFB1, most of them oxidoreductases, but none showed homology with the AFB1-degrading enzymes of M. smegmatis. The largest difference was shown by the flavin-dependent oxidoreductase HG717_03460 (5.5-fold increase), but other NAD(P)/FAD-dependent oxidoreductases were also found among the up-regulated

proteins. Our future plans include heterologous expression of these oxidoreductases identified by proteome analysis in E. coli and examination of their AFB1 degradation.

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Environmental Microbiology and Biotechnology, -

Genome and proteome analyses of „master of biodegradation" Rhodococcus erythropolis NI86/2

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Rhodococci are Gram-positive, high GC content, aerobic nocardioform actinomycetes, inhabiting many diverse niches. Members of the genus Rhodococcus are regarded as masters of biodegradation as they can transform and degrade a wide range of xenobiotic compounds. One of the first rhodococcus strains reported to utilize/degrade xenobiotics is the R. erythropolis NI86/21 strain, which was isolated from an EPTC herbicide treated soil in Hungary as thiocarbamate herbicide degrader. The biodegradation capacity of the Ni86/2 strain is indicated by the fact that beside herbicides it is one of the most active aflatoxin, zearalenone and T2 toxin-degrading microbes. Also this strain was the source of the first transposon mutagenesis plasmid for manipulating rhodococci.

The genome of our model organism, Rhodococcus erythropolis NI86/21 has been sequenced on Illumina and PacBio platforms. The genome of the NI86/21 strain is among the largest R. erythropolis genomes (8.0 Mb) and it could be assembled into 16 contigs, of which the two largest, the 4.7 and the 2.07 Mb contigs together with a 121 kb DNA fragment constitute the chromosome. Contigs 7, 8 and 16 are circular plasmids with 105, 105 and 5.9 kb sizes, respectively. We also found evidence by homology searches, that the rest of the contigs belong to at least two linear plasmids, since telomere binding and terminal proteins were encoded at the C-terminus of the 255 and 73 kb contigs. We found partial or full lenght sequences of the novel R. erythropolis transposon (Tn7706) of 19 kb size in most of the contigs at N- and/or C-terminal positions, or internally.

A genome analysis experiment with the Alien hunter search machine found large DNA fragments integrated into the chromosomal DNA. The largest alien DNA fragments were encoded on the 4.7 Mb chromosomal contig in the regions of 1 – 280000, 3407347 – 3572842 and 4627092 – 4735238 positions. These sequences show homology to linear plasmids, transposons, phages and insertion elements, and only a very low percentage of alien DNA at the C-terminal end showed homology to chromosomal DNA. Most of these alien DNA fragments showed no homology to known sequences on DNA level, however randomly

selected ORFs of these regions showed homology to filogenetic neighbors like R. quinsengi, R. opacus, Mycobacterium frederiksbergense, and Gordonia, Williamsonia and Streptomyces strains. DNA fragments with similar features were also encoded on the linear megaplasmids (contig 3 and 4), whose certain DNA stretches showed homology to M. frederiksbergense.

All together we found that approx. 1.5 Mb of the genome was acquired by horizontal gene transfer (HGT). These HGT DNA regions seem transcribed with the same frequency as the chromosomal genes, revealed by Lc-MS/MS based proteome analysis of R. erythropolis NI86/21 cells grown at normal culture conditions in LB medium. It worth noting, that those genes, which made this microbe unique (ThcB and ThcC genes taking part in thiocarbamate and triazin herbicide degradation, the second copy of 20S proteasome, the IS1415 insertion element which was developed to a transposon and the small plasmid that was used to create the shuttle vector pFAJ2574) were encoded in HGT regions.

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Endocrine disruptor-degrading bacteria isolated from effluent wastewater and Danube water-based microcosm

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Keywords: endocrine disruptor molecules, microbial degradation, Pseudomonas fulva

Endocrine disruptors are potential environmental contaminants that can cause toxicity in aquatic ecosystems. In March 2023, ninety-eight bacterial strains were isolated and identified from purified sewage effluent and a Danube water-based microcosm using a selective medium containing bisphenol A, 17β-estradiol, and nonylphenol as the sole carbon source.

All isolated heterotrophic bacteria belonged to Pseudomonadota with the exceptions of Paenibacillus lautus, Bacillus cereus and Bacillus paramycoides. Other isolated bacteria belonged to the Gammaproteobacteria (members of Citrobacter, Enterobacter, Escherichia, Klebsiella, Kluyvera, Leclercia, Raoultella, Shigella. Acinetobacter, Aeromonas, Pseudomonas, Rahnella, Phytobacter, Kosakonia genera). Among Alphaproteobacteria, genera Novosphingobium, Sphingobium and Ensifer were found, while the betaproteobacterial representatives were assigned to the genus Burkholderia.

The degradation capacities of the strains for bisphenol A, 17β-estradiol and nonylphenol were tested using three types of selective media. containing the endocrine disruptor molecules as sole carbon sources at 700 mg/L concentration.

Following the assessment of their degradation capacity, non-pathogenic bacteria, Pseudomonas fulva HF16, Pseudomonas nicosulfuronedens I12A, Pseudomonas nitritireducens I1B and Pseudomonas vancouverensis ST6 were selected for further analysis.

Degradation capacity of Pseudomonas fulva HF16 was checked in liquid media containing the compounds at a concentration of 50 mg/L, respectively. Following a two-week period, quantitative analysis was done using the Dionex Ultimate 3000 UHPLC system connected to an Orbitrap Q Exactive Focus Mass Spectrometer equipped with electrospray ionization. Pseudomonas fulva HF16 strain could utilize the investigated endocrine disruptors: bisphenol A by 34%, 17β-estradiol by 52%, and nonylphenol by 54%.

Further studies are necessary using lower concentrations and exploring other, non-pathogenic environmental bacteria for their potential to degrade organic micropollutants.

Environmental Microbiology and Biotechnology, -

Tracking SARS-CoV-2 variants in wastewater

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Epidemiologists have been using information obtained from wastewater for decades for the monitoring of several parameters of public health concern. The wastewater serves as a community fingerprint, reflecting the spatio-temporal changes in the health status of the serviced populations. Following the early monitoring of poliomyelitis virus, several methods have been developed to widen the “tool-box” available for wastewater-based epidemiology (WBE). This scientific field gained increased interest during the COVID-19 pandemic, when it was used to monitor the severity of pandemic waves. WBE also served as a reliable data source during the pandemic for public health decision makers by identifying the circulating SARS-CoV-2 variants and their relative abundances. The rationale of variant detection and quantification is that distinct lineages of the same pathogen can call for different action depending on their characteristics (virulence, transmissibility, immunity evasion). The simultaneous determination of different SARS-CoV-2 variants in the sewage and the estimation of their relative abundance required the development and adaptation of several next generation sequencing (NGS) techniques and bioinformatics packages/programs.

In this study, the genetic material of SARS-CoV-2 for sequencing was obtained from concentrated wastewater samples using an in-house protocol. This included the pre-treatment of the samples by centrifugation to remove suspended solids. Samples were concentrated by flat-sheet ultrafiltration. RNA was extracted with a commercial silica-based technique. Libraries of cDNA were generated prior to for sequencing. The cDNA libraries from the different samples were sequenced using Illumina MiSeq or Illumina NextSeq devices based on the sample characteristics. The evaluation of the sequencing results required the use of several bioinformatics programs. The preprocessing of the sequences was performed by FastP. Reads, which passed the quality control were aligned by the BWA-MEM software to the SARS-CoV-2 reference genome. During the following steps, several Samtools functions were applied to modulate the alignments. The multifunctional iVar package was used for both the primer

trimming and the detection of genome variants. Finally, Freyja was utilized during the accurate quantitative estimation of the variant frequencies above 1% variant abundance. Routine monitoring based on these methods has been taking place since the August of 2023. Since then, we were able to track the emergence of the BA.2.86 lineage and later of its sub-lineage JN.1. The obtained results were in agreement with the clinical data and provided reliable complementary information for public health decision making to other conventional surveillance techniques. This research was carried out with the financial support of EU-WISH (No. 101140460) project.

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Biotransformation of diclofenac by Stenotrophomonas humi strain DIC_5 and ecotoxicological assessment of the residual metabolites

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The increasing concentration of diclofenac, a widely used nonsteroidal anti-inflammatory drug, in freshwater ecosystems has become a pressing environmental issues for humanity. In this study, the diclofenac degradation ability of the bacterial isolate Stenotrophomonas humi DIC_5 strain was investigated. This bacterium was able to eliminate 75.1% of diclofenac at an initial concentration of 1.5 mg/L within 8 days in the presence of glucose (3.0 g/L). During this process, nitro-diclofenac was identified as the metabolite produced, and its concentration in the bacterial culture medium increased significantly from the 7th day of the experiment, meanwhile the concentration of diclofenac decreased. Ecotoxicological tests with both Aliivibrio fischeri and zebrafish embryos showed that diclofenac biotransformation products were non-toxic to the test organisms at the concentrations used in the work. However, in the absence of the xenobiotic (diclofenac), bacterial metabolites in the medium were more toxic to the test organisms than the biodegradation samples. This could mean that the presence of the diclofenac signal reduced the toxic effects caused by the bacteria. Our work could raise concerns about the use of microorganisms in biodegradation experiments, especially when xenobiotics are applied at intermediate or environmentally relevant concentration ranges.

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The comparative analysis of electroactive *Shewanella* isolates

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Members of the genus Shewanella are Gram-negative bacteria which due to their efficient electron transfer capabilities are potential candidates for green energy production, water treatment, and biosensor applications based on microbial fuel cell (MFC) technology. One of the less-described species of this genus is Shewanella baltica whose members are mostly isolated from contaminated freshwater and marine food sources. In our work we identified five different S. baltica isolates using the MALDI-TOF method. Further investigations using sequenced genomes revealed that one of these isolates belonged to the less-studied species Shewanella morhuae while the other was identified as Shewanella putrefaciens. During our research we compared these isolates based on various properties. Based on morphological differences two phenotypes could be distinguished and some differences could be also revealed in their biochemical properties with the VITEK 2 method. The extracellular enzymatic activities of the strains were similar showing moderate protease and lipase activities and a lack of starch degradation capability. Regarding practical applications, the strains demonstrated effective electroactivity in phenotypic tests using Reactive Black 5 differential medium and a tungsten nanowire reduction method. In terms of biofilm formation, they proved effective on polystyrene (96-well format), glass (reaction tubes) and carbon cloth (Scanning Electron Microscopy). Based on these properties, the strains are potential candidates for further studies aimed at evaluating their efficiency and application in microbial fuel cells (MFC).

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Environmental Microbiology and Biotechnology, -

The happy marriage of Methanogenic Archaea and Anaerobic Fungi for symbiotic gas fermentation

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Degradation of lignocellulose-rich material into biogas is an attractive strategy to ameliorate growing renewable energy demands and mitigate greenhouse gas emissions. Lignocellulosic biomass is the largest renewable organic resource on Earth (1) and do not trigger “food or fuel” conflicts. It is composed of interwoven cellulose and hemicellulose fibres glued together by anaerobically recalcitrant lignin (2). That is why the microbial community of the biogas reactor is inefficient in lignocellulose deconstruction leaving the more easily digestible polymeric sugars behind. Microbial pre-treatment utilizing the fibre degrading potentials of aerobic fungi may be a cheap and efficient approach although not without challenges (3). Anaerobic fungi (AF) from the phylum Neocallimastigomycota break down lignocellulose with high efficiency. AF are ubiquitous in the rumen and significantly contribute to the nutrition of herbivorous

animals (4). AF anchor themselves to the plant material and crack the fibres mechanically by their growing rhizoids (5). To exploit this feature, we need to understand the ideal conditions for their optimal operation. Moreover, their symbiotic, prokaryotic intimate partners, participating in the subsequent utilization of the degradation products, should be identified and the successful coexistence of the interkingdom mates should be established.

We therefore isolated AF and their methanogenic partners from anoa, elephant and mouflon. The lignocellulose degradation efficiency using pure AF culture and AF-methanogenic co-cultures were tested.

A 14-day incubation with AF increased the cumulative biomethane yield in 20 days. Pretreatment with AF considerably improved the degradability of lignocellulosic substrates. Concomitant and proportional production of high-value organic acids commodities were determined. Correlations between the fermentation products and key enzyme activities were recognized.

In summary, AF-methanogen symbiotic teams are effective in enhancing lignocellulose degradation, successfully increasing gas fermentation and organic acid production.

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Environmental Microbiology and Biotechnology, -

Microplastic as environmental vectors for seweage originated antibiotic-resistant Pseudomonas aeruginosa strains

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Due to their slow degradation rates, plastics break down into microplastics (MPs), which persist in the environment for years, interacting with chemical compounds and microorganisms. Consequently, MPs provide new ecological niches for bacterial and eukaryotic cell colonization, and biofilm formation and act as potential vectors in pathogen transmission. This

new environmental niche is known as the "plastisphere," which is considered a hotspot for antimicrobial resistance.

Wastewater is a known source of microplastics: MPs originating from wastewater often enter the environment, offering abiotic surfaces for bacterial biofilm formation. The aim of this research was to isolate biofilm-forming and antibiotic-resistant opportunistic pathogen Pseudomonas aeruginosa strains from wastewater treatment plants (WWTPs), including raw and treated wastewater, sludge, and the surface of microplastics, assess their antibiotic resistance profiles, and determine the role of MPs in their transmission.

According to our results, in case of wastewater samples from Hungarian WWTPs, P. aeruginosa was detectable in 66 of the 87 samples (75.86%). Antibiotic susceptibility testing revealed that most strains were commonly resistant to antibiotics, with five strains classified as multidrug-resistant (MDR), showing resistance to at least three classes of antibiotics. Biofilm formation assays demonstrated that the examined strains exhibited moderate to strong biofilm formation on polystyrene surfaces.

This study findings highlight the significance of antibiotic-resistant P. aeruginosa in the wastewater treatment process and confirm the species' variable ability to form biofilms on certain polymer surfaces. Our results could have future applications in the fields of wastewater treatment and water safety.

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Environmental Microbiology and Biotechnology, -

Investigating the solo and combined effects of beauvericin and different antibiotics on Pseudomonas aeruginosa strains

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According to the 2023 World Mycotoxin Survey, beauvericin (BEA) was the second most frequently detected mycotoxin, with the prevalence of 67% among 4601 tested samples, based on 78 countries and 243853 analysis points. Despite its widespread occurrence, BEA - as an emerging mycotoxin - is not under food safety regulation as further data are required to determine its chronic toxicity effects. Moreover, BEA has shown many promising bioactive properties assigning it as a potential candidate for pesticide and medicine development, as its antiviral, antibacterial, antifungal and insecticidal effect have been published on numerous occasions. The present study focuses on the antibacterial activity and potentiation effect of BEA, which has been demonstrated in the literature. BEA is effective against a wide range of gram-negative and gram-positive bacteria, however, there is lack of consensus regarding the concentrations at which the substance exerts its antibacterial effect. In case of Pseudomonas aeruginosa, the minimum inhibitory concentration (MIC) has been defined between 1 to 80 ppm in the literature. Our preliminary experiments aimed to examine the effects of BEA on the

growth and antibiotic resistance profile of representative P. aeruginosa strains (ATCC 27853, ATCC 10145 and ATCC 15442). In our preliminary experiment, we dissolved BEA in DMSO, at the 1.2% DMSO concentration tolerated by the strains. The solubility of BEA in aqueous medium was 12.5 ppm, up to this concentration no significant toxic effect was measured, therefore, the MIC of BEA on the examined strains should be above this concentration. Based on our preliminary experiment, our objective was to test the potentiating effect of BEA on 10 different antibiotics (ceftazidim, cefepim, imipenem, doripenem, piperacillin, aztreonam, netilmicin, ciprofloxacin, colistin, levofloxacin). The results demonstrated a considerable potentiation effect for the antibiotic colistin in all strains. Our results provide the first insight on the antibacterial activity of BEA on the critically important opportunistic species P. aeruginosa and at the same time reveal its potentiation effect in combination with medically important antibiotics.

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Environmental Microbiology and Biotechnology, -

Novel energy conserving technology: conversion of gaseous compounds to bioCH4 with mixed anaerobic microbial consortia

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The accelerating global climate change leads to increased renewable energy demand. These needs are met either by burning fossil fuels or through the growing implementation of "green", i.e. renewable technologies. The uncontrolled exploitation of fossil fuels depletes the available resources at dangerous pace accompanied by the emission of harmful “greenhouse” gases into the atmosphere, contributing to inevitable changes on the global climate. Gasification of biomass results in a gas mixture that comprises hydrogen (H2), carbon monoxide (CO), and carbon dioxide (CO2). This so-called “synthesis gas (Syngas)”, can be utilized in several biotechnological conversion processes [1].

Efficient solar energy storage alternatives, such as photovoltaics have developed extensively in spite of the serious challenges due to the inflexibility of the electricity grids. The “Power-to-Gas” (P2G) technologies provide convenient solution converting the excess “green electricity” to H2. However, the currently available H2 storage and transportation technologies are inefficient and expensive, which call for finding suitable, more cost-effective energy carriers via the conversion of the “green” H2 [2].

The integration of Syngas fermentation with the P2G concept further enhances its applicability within the renewable energy landscape with the help of the anaerobic biogas fermenting microbes. Anaerobic methane (CH4) forming communities offer an alternative solution for both the disposal of CO2, CO from the Syngas and for the utilization of H2 in CH4 production. The diverse methanogenic metabolism of the mixed community is capable to utilize these gases via the bioconversion activity of the carboxydotrophic methanogens [3]. Addition of bio-H2 increases the reducing capacity of the system, which generates excess CH4 and diminishes the unwanted CO2.

Our main goal has been understanding the microbial response to the Syngas fermentation of various H2, CO2 gas mixes with and without the added CO. The adaptation of the microbial community to this environment was followed by metabolic product determination and metagenomic analysis of the evolved communities.

The proposed strategy successfully integrates the hazardous Syngas elimination with the renewable “green power” conservation into alternative energy carrier (bio-CH4) [4]. The biogas producing microbial community serves as an excellent catalyst of the P2G-derived H2, and the industrial by-products CO, CO2 into bio-CH4. Thus the biogas fermentation residue reservoirs may acquire an entirely new function in the gas fermentation and bioconversion technology in large-scale industrial applications.

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Molecular and Clinical Microbiology , -

Cutibacterium acnes triggers skin region-specific innate immune memory events in human keratinocytes

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External insults can activate epithelial cells at the interfaces between the body and the environment. The resulting immune and inflammatory activation may have a lasting impact. The skin microbiota is in constant contact with the keratinocytes, which utilize pattern recognition receptors to detect microbes.

The study aimed to investigate whether Cutibacterium acnes (C. acnes), a member of the skin microbiota, can induce a persistent inflammatory memory in keratinocytes, known as innate immune memory (IIM).

We performed initial training with C. acnes, followed by a five-day resting period and a secondary induction with Pam3CSK4 (TLR1/2 agonist) in normal human epidermal keratinocytes from breast (NHEK-B) and abdominal (NHEK-A) surgery.

The differential expression of the immune effectors TNFα and IL-8 indicates regional differences in NHEK-B and NHEK-A cell samples, with higher expression levels in NHEK-B cells and the lower expression in NHEK-A samples suggest region-specific innate training and tolerance events, respectively.

Transcriptome analysis of C. acnes-trained cells NHEK cells revealed significant differences in the residual gene expression pattern long after microbial induction. While NHEK-A cells exhibited significant alterations in gene expression associated with tissue repair, NHEK-B cells demonstrated diminished oxygen-sensing capabilities, potentially attributed to elevated levels of oxidative stress and inflammation. Following Pam3CSK4 induction, NHEK-A cells exhibited modifications in skin development, signaling pathways, and extracellular matrix formation, in contrast to NHEK-B cells, which exhibited altered inflammatory responses.

These differences may be attributed to the differences in epigenetic regulation of NHEK-B and A cells. The baseline global 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) content of genomic DNA was reduced in trained, uninduced NHEK-B cells compared to NHEK-A. This observation was supported by the region-specific mRNA expression variances of the TET and DNMT genes.

The C. acnes training had an impact on the metabolism of NHEK cells. A reduction in lactate production and an elevated NAD+/NADH ratio were observed in untrained compared to trained NHEK-B cells, indicating decreased glycolysis. Treatment with Pam3CSK4 resulted in an increase in lactate production and a decrease in the NAD+/NADH ratio, specifically in the C. acnes-trained NHEK-B cells, suggesting an enhanced glycolysis.

These findings demonstrate that C. acnes induces region-specific IIM in keratinocytes, with underlying signaling, epigenetic, and metabolic changes contributing to the distinct responses across skin regions.

Molecular and Clinical Microbiology , -

Exploring the Role of Chlamydia Infections in NETopathic Airway Inflammation: A Mouse Model Study

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Chlamydia pneumoniae is thought to worsen asthma by triggering both airway eosinophilia and neutrophilia, thereby increasing the severity of airway disease and asthma symptoms. Additionally, the persistence of C. pneumoniae can provoke a strong inflammatory response, leading to increased cytokine levels and airway remodelling in affected individuals. Neutrophilic extracellular DNA (dsDNA) originates from neutrophil extracellular traps (NETs), which are formed as a response to various pathogens. These web-like structures capture pathogens and expose them to antimicrobial actions. NETs can thicken mucus, intensify airway inflammation, and cause tissue damage, contributing to the pathogenesis of asthma, COPD, and cystic

fibrosis. NETs also serve as a platform for complement activation, such as C5a, and stimulate further cytokine production.

Given the lack of available data, we aimed to explore the potential link between Chlamydia infections, NETosis, and airway inflammation. We infected Balb/c mice with C. muridarum or C. pneumoniae, while a control group was left untreated, and the mice were sacrificed seven days post-infection. Lung tissue was processed for proteomic analysis following bronchoalveolar lavage (BAL). Proteomic analysis identified 3,102 proteins for C. pneumoniae and 4,270 for C. muridarum, which were subjected to gene set enrichment (GSEA) and KEGG analysis using the R clusterProfiler package. In C. pneumoniae, 169 proteins were significantly upregulated and 446 downregulated, whereas in C. muridarum, 324 were upregulated and 435 downregulated. Pathway analysis indicated that C. pneumoniae activates key components of lytic NETosis, including neutrophil elastase (NE), myeloperoxidase (MPO), and cathelicidin (LL37) via caspase 1 and gasdermin D (Gsdmd) signalling. In contrast, C. muridarum exhibited C5aR1 activation and dependency on PAD4 and calcium signalling, resulting in HDAC activation. Our data suggest that C. pneumoniae predominantly acts on the classical pathway with C1q and C5aR activation, while C. muridarum activates the alternative pathway via C3 and properdin.

To quantify dsDNA, we performed a PicoGreen assay and found significantly higher dsDNA levels in infected mice compared to controls. In vitro, C. muridarum-treated bone marrow-derived neutrophils (BMDNs) showed significantly higher dsDNA levels than PMA-treated or untreated cells.

Our preliminary findings suggest that different Chlamydia species induce NETosis and complement activation through distinct mechanisms, indicating that only C. pneumoniae should be used to model airway NETosis. Since infection-related NETosis can affect disease severity in asthma and cystic fibrosis, our data could inform future research on NET inhibitors.

Molecular and Clinical Microbiology , -

The double-stranded RNA analogue polyinosinic–polycytidylic acid and IFN-lambda2 cooperatively stimulate autophagy

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Autophagy is a cellular catabolic process implicated in the early cellular defense mechanisms in infections. Viral infections can provoke autophagy, while some gene products can function as inhibitors of this antimicrobial pathway. The replication of several viruses results in the formation of double-stranded DNA (dsDNA) or dsRNA intermediates. These replication intermediates are detected by pathogen recognition receptors, which activate cellular defense responses. dsDNA/dsRNA are powerful inducers of interferons (IFNs) exerting antiviral and antiproliferative effects. The course and outcome of viral infections are greatly influenced by the concerted action of dsDNA/dsRNA replication intermediates and interferons. Since the combined effect of IFN-lambda2 and dsRNA on autophagy has not yet been investigated, we evaluated the autophagic activity in the 92-1 melanoma cell line treated with the viral mimetic polyinosinic–polycytidylic acid [poly(I:C)] and IFN-lambda2. The levels of LC3B-II and autophagic flux were determined by western blotting. The intracellular localization of LC3B was

measured by immunofluorescence assay. Cytoplasmic acidification was investigated by acridine orange staining. The activation levels of signaling pathways implicated in autophagy regulation were evaluated by using a phosphokinase array. The results showed that the combined treatment with poly(I:C) and IFN-lambda2 elicited a significant increase in the level of LC3B-II. Furthermore, these data were used to calculate the formation, degradation, and net turnover ratios. The results showed that the autophagosome formation ratio was increased, whereas the autophagosome degradation ratio was decreased significantly in cells treated with poly(I:C) and IFN-lambda2. Thus, the combined poly(I:C) and IFN-lambda2 treatment elicited a significant increase in the net autophagic turnover rate. The average number of LC3B-positive vacuoles per cell in the cultures treated with poly(I:C) and IFN-lambda2 was significantly higher than in the control cultures. The combined poly(I:C) and IFN-lambda2 treatment increased the average number of acidic vesicular organelles. Our studies have shown that the levels of phospho-PLCG1 (Y783), phospho-STAT-2 (Y689), phospho-STAT5a/b (Y694/Y699), p38alpha MAPK (T180/Y182), JNK1/2/3 (T183/Y185, T221/Y223) and some phospho-SRC family members were increase, whereas AKT1/2/3 (S473 and T308), PRAS40 (T246) and p70S6K (T389 and T421/S424) were hypo-phosphorylated in cells treated with poly(I:C) and IFN-lambda2. The combined poly(I:C) and IFN-lambda2 treatment appears to cause a pro-autophagic shift in signaling that regulates autophagy. In summary, these data indicate that combined poly(I:C) and IFN-lambda2 treatment differentially regulates the phosphorylation status of signaling molecules implicated in autophagy regulation, thereby increasing the lipidation of LC3B-I and stimulating autophagosome formation.

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Industrial Microbiology – Fungal Industrial Biotechnology, -

Trapped in steel: effect of Mn2+ on citric acid production by Aspergillus niger

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Achieving high yields of citric acid requires a unique combination of culture conditions, with the deficiency of manganese(II) ions in the growth medium being especially crucial. Concentrations exceeding 5 µg/L (= 5 ppb) result in a reduction of around 25% in the final citric acid yield. Because of its characteristics, this organic acid finds utility across a spectrum of industrial sectors, spanning from food and beverage to detergent and pharmaceutical industries. The predominant method for citric acid production involves large-scale industrial fermentations utilizing the filamentous fungus Aspergillus niger. When compared to alternative hosts, A. niger stands out for its ability to achieve remarkably high yields, with potential outputs reaching up to 95 kg of citric acid per 100 kg of sugar.

Technical-scale production of citric acid predominantly uses stainless steel tank fermenters. However, glass bioreactors, commonly used for process development, also incorporate stainless steel components, where manganese serves as a crucial alloying element. Our study reveals that manganese(II) ions leach from these bioreactors into the growth medium during citric acid fermentations. This leaching phenomenon leads to alterations in fungal physiology and morphology, resulting in a significant decrease in citric acid yields. The extent of manganese(II) ion leaching depends on factors such as fermentation duration, the acidity of the culture broth, and the sterilization method employed.

Moreover, CexA is the main citrate exporter of A. niger. The citric acid production was examined in overexpression mutant strains of cexA under manganese deficiency and sufficient conditions. This leads to citric acid accumulation even in the presence of high manganese(II) ion concentrations. Additionally, the impact of CexA on fungal morphology was elucidated through microscopic analysis.

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Industrial Microbiology – Fungal Industrial Biotechnology, -

The Robust Fungus: The Importance of Aspergillus niger in Biotechnology

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Microorganisms have been used by mankind for millennia in various fields. Modern fungal biotechnology, specifically the controlled cultivation of fungi in bioreactors, was born in 1919 when Pfizer began exploiting Aspergillus niger's ability to overproduce and secrete citric acid. Aspergillus niger is a widely known fungus with significant importance in the food industry, biotechnological applications, and medicine. Its robustness is particularly noteworthy, as it can survive and grow under extreme environmental conditions, making the fungus highly resistant.

Aspergillus niger can grow within a wide temperature range, typically between 6°C and 47°C. This broad range allows it to thrive in various environments. The fungus is also capable of growing in low pH environments (as low as pH 1-2), which provides it with extraordinary resistance to acidic conditions. This trait is especially useful in industrial applications, where it is often cultivated under acidic conditions.

The fungus can utilize a wide range of substrates as energy sources, including various carbohydrates, organic acids, and alcohols. This capability allows it to grow on different organic materials. These combined characteristics make Aspergillus niger extremely robust, making it widely applicable in various industrial processes, such as the production of enzymes, organic acids, and other biotechnological products.

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Industrial Microbiology – Fungal Industrial Biotechnology, -

Albitech Ltd. has been involved in algae research for 15 years: the creation of a collection of strains, algae products for agriculture and recent innovations in algae biotechnology.

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Our core business is the application of green solutions in agriculture. The use of algae foliar fertilizers and soil inoculants offer the possibility of phytostimulating arable crops. Dense living suspensions of microalgae or their special extracts can stimulate plant growth and yield. In addition, single-cell algae can also be used for plant protection purposes, e.g. by exploiting their metabolites against plant pathogenic bacteria. In the initial phase of our research, we created algal isolates from different lake water layers and soil surfaces for our algal strain collection. Cultures were purified and identified by 18S rRNA sequencing in collaboration with the Department of Microbiology of ELTE. Thus, in some cases new species were discovered. Bioassays were set up to detect their plant hormone production and analytical HPLC-MS/MS methods were used to detect the concentration of phytohormone produced by the selected isolates - collaboration with ELTE and BLRI. Our research has shown that microalgae cultures of the Balaton isolate Scenedesmus - Algafix foliar fertilizer- have a hormone-like stimulating effect similar to plant hormones. A soil erosion reducing preparation of soil algae - AlgaTer - has been developed: it has been shown that the biological soil crust tissues of the green alga Klebsormidium filamentous form a biofilm layer around soil particles, reducing soil surface erosion, helping to retain soil moisture and thereby producing hormones that promote plant growth. In plant experiments, we have demonstrated positive effects on plant growth, flowering and yield – collaboration with ABC and NEBIH. We have also tested extracts of certain algal strains and demonstrated antimicrobial effects against human and plant pathogens. Communication between microalgae and soil bacteria has been demonstrated by microfluidic methods-collaboration of RCB. Microalgae possess specific pigments whose antioxidant activity is suitable for animal feeding or even human physiological studies. Their specific PUFA and lipid content differs from eukaryotic plants, and have provided human neurophysiological test results that raise the possibility of producing food supplement algae extracts. Of course, setting optimal conditions for algal cultures, providing adequate light to the photobioreactor and optimising the photosynthetic activity and growth of the cultures are constant challenges for our algae plant. With our new flat-panel photobioreactors, we are able to produce strain cultures of different algae species at high densities. With optimum light intensity, carbon-dioxide dosing and automated pH and heat control, we aim to produce the highest cell counts and algal solids - 3.0 x 107 -3.0 x 108 cells/ml and 2.0 -20 g d.m./l - which is already competitive with conventional fermentation techniques. The ability of algae to produce large amounts of cell concentration and/or biomass, similar to yeasts or even bacteria, could represent a major breakthrough in the biotechnology industry.

Industrial Microbiology – Fungal Industrial Biotechnology, -

Examination of Yarrowia divulgata 1485 pigment production

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Yarrowia lipolytica is an increasingly widely used and studied microorganism in industry, as it is used in bioconversion steps, biodegradation processes and in the production of various products (citric acid, polyols). The yeast Yarrowia lipolytica naturally produces pyomelanin, a metabolite derived from L-tyrosine, by accumulating in the extracellular space. Pyomelanin is a brown pigment that provides UV protection and has antioxidant activity, so it is often used in cosmetic and pharmaceutical products.

In my research, I aimed at enhancing the pigment production of the closely related strain Yarrowia divulgata 1485, Y. lipolytica. First, I determined the optimal tyrosine concentration

(g/l) using by microtitre plate. Because of the high throughput of this method, I was able to perform all the adjustments in 3 parallel runs simultaneously. After the determined tyrosine concentration, I tried to enhance pigment production by adding various other components, amino acids. The following components were tested: glycine, l-phenylalanine, CuSO4, lactic acid. After these tests, I increased the scale up with the medium that proved to be the best, using Erlenmeyer's flasks and a 300 ml fermentor. Further objectives include the investigation of the effect of several amino acids according to the Plackett-Burman design and scale-up, where I would like to perform pigment fermentation in a 1 l fermentor.

Industrial Microbiology – Fungal Industrial Biotechnology, -

An ecophysiological study of a Klebsormidium strain with biotechnological applications

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Species of the Klebsormidium genus play a crucial role in forming biological soil crusts (BSCs), which contribute to soil surface stabilisation, improve soil structure, and enhance water retention. Due to their relatively high brassinosteroid content and crucial role in soil ecosystems, Klebsormidium species hold a great potential for biotechnological applications. In our study, we investigated temperature dependence of the photosynthetic characteristics and brassinosteroid (BR) content during of a Klebsormidium strain isolated from a Hungarian cave. We assessed the strain's temperature and light optimum through oxygen yield and pulse-amplitude-modulated fluorescence measurements. We also evaluated the specific growth rate of the strain from 10 to 40°C. Based on our results we can conclude that this microalgae could perform net photosynthesis between 5 and 45°C. The maximal photosynthetic activity was observed between 30 to 40°C and 35 to 40°C when it was determined by oxygen yield and chlorophyll fluorescence measurements, respectively. We also found that the strain exhibited a broad growth temperature range, with an optimum of 20 to 25°C. As anticipated, the cellular BR levels in this Klebsormidium strain were strongly influenced by temperature, with the maximum levels occurring during temperature stress. The strain showed high light utilization factors (α) and low light adaptation parameters (Ik) regardless of the temperature. Thus, despite its cave origin, the examined Klebsormidium strain can grow and photosynthesize over a wide temperature range, underscoring its potential for biotechnological applications. Albitech Biotechnological Ltd. has developed a soil inoculant product, AlgaTer, which reduces soil erosion by exploiting the properties of this algal strain. The strain forms a biofilm around soil particles, which reduce surface erosion, and retains soil moisture. Positive effects on plant growth, flowering and yields have been demonstrated in laboratory experiments and field trials - in collaboration with the Institute of Genetics and Biotechnology (MATE) and the National Food Chain Safety Office.

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Industrial Microbiology – Fungal Industrial Biotechnology, -

Manganese deficiency triggers citrate export via cexA upregulation in Aspergillus niger during high-yield citric acid fermentation

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Citric acid (CA) is amongst the most important bulk products of industrial biotechnology. Being the denominator intermediate of the ubiquitous citric acid cycle, CA occurs in almost every living organism. The discovery of its accumulation by the filamentous Ascomycete fungus Aspergillus niger led to a rapid development of a fermentation process which accounts for almost all of the world's CA production. Currently, CA is produced by large-scale (> 200 m3) submerged fermentation technology employing stirred, stainless steel tank reactors and selected mutants of A. niger.

Citric acid overflow requires a unique combination of unusual nutrient conditions: very high concentrations of H+, dissolved oxygen and initial carbon source, as well as growth-limiting concentrations of nitrogen, phosphate, as well as iron, zinc, copper, and manganese ions. Of all the metal ions, adjusting the correct levels of Mn(II) is the most critical, as concentrations as low as >5 ppb already reduce CA accumulation by 20%. The deleterious impact of Mn(II) ions on the A. niger CA overflow is called "the manganese effect".

CA export from the cytosol significantly increases in mycelia grown under Mn(II) ion deficiency. To understand how Mn(II)-deficiency prompts CA overflow, we compared the transcriptome of the citric acid hyper-producer A. niger strain NRRL2270 at three time points during citric acid production at Mn(II) ion deficient and Mn(II) ion sufficient conditions: at 24 hrs, when CA just starts to accumulate; at 48 hrs, when phosphate is already exhausted; and 72 hrs where CA accumulation has reached the maximal production rate.

The A. niger NRRL3 genome lists 11.846 genes. Of these genes, 963 (8.1%) were identified as differentially expressed under the present conditions, i.e., whose transcript had a mean TPM (transcripts per million) value higher than 10, and whose transcription differed between manganese deficiency and manganese sufficiency by a log2 greater than two or smaller than minus two at $p < 0.05$. Among the 963 differentially expressed genes, 390 were upregulated and 573 were downregulated under manganese deficiency.

We observed that Mn(II) deficiency triggers up to 110-fold increase in the cexA transcript that encodes the citrate exporter, suggesting that triggering cexA transcription could be a crucial factor in the necessity of manganese limitation. To test this, we created a cexA-overexpressing strain where the native cexA locus was replaced with a glaA:cexA construct that expresses cexA under the glucoamylase promoter. This indeed led to high and Mn(II)-independent levels of cexA transcription. In the presence of 5 ppb Mn(II) ions, both the overexpressing and the parental strains accumulated high concentrations of CA. In contrast, the presence of 100 ppb Mn(II) ions in the growth medium resulted in a final citric acid concentration of only 40 g L-1 in the parent strain, while the overexpressing strain produced up to 100 g L-1 citric acid, corresponding to 83% of the concentration that accumulated under manganese limitation. We conclude that triggering of citrate export is a key event caused by manganese deficiency in A. niger during CA fermentation.

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of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund.

Industrial Microbiology – Fungal Industrial Biotechnology, -

From origin to evolution: the story of alternative oxidase in Aspergillaceae

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Alternative oxidase (Aox) is a branched mitochondrial terminal oxidase, which bypass the Complex III. and IV. Aox accepts electrons directly from ubiquinol and reduces oxygen to water without contributing to the proton gradient used for ATP synthesis. Aox is known to have various functions, including helping cells to manage stress conditions, regulating cellular metabolism, and maintaining redox balance. In certain organisms, such as plants, it can play a role in preventing excessive reactive oxygen species production under stress conditions.

Aox is near ubiquitous in fungi, yet the presence of multiple aox genes is uncommon. However, a second aox gene (aoxB) is present in some taxa of Aspergillaceae. Paralogous genes typically originate from duplication events and are passed down vertically. We offer evidence of four separate duplication events along the lineage that resulted in aox paralogues (aoxB) in contemporary Aspergillus and Penicillium taxa. In certain species, three aox genes are co-expressed, yet there are entire sections and series within Aspergillus that evidently loss of transient aoxB content. Within the subgenus Nidulantes, we have identified seven instances of independent aoxB gene loss and two instances of gain. The paralogous clades originate from widespread aoxA parent genes but never replace them, aoxA remains permanent across filamentous fungi.

The investigation of alternative oxidase genes is crucial for a deeper understanding for citric acid production or clinical aspects. Fermentation occurs based on highly complex biochemical relationships, wherein the aox gene plays a crucial role. Understanding a gene well significantly contributes to strain development or possible clinical treatment, the public genetic databases can provide valuable information regarding its origin and evolution.

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Industrial Microbiology – Fungal Industrial Biotechnology, -

Alternative Oxidase in the Biotechnology: Is less Sometimes More?

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The alternative oxidase (Aox) can provide for an alternative respiratory pathway, serving as an additional component alongside the main respiratory chain in plants, fungi, and certain animal cells. It has been well studied in plants, but there is also a growing interest in fungi.

The Aspergillaceae family, which includes genera such as Aspergillus and Penicillium, harbors several platform species crucially important in biotechnology (e.g., A. niger, A. terreus, A. oryzae, P. chrysogenum), whose alternative oxidase function is in most cases essential for the accumulation of the metabolic products. The exact biochemical function of this protein is not yet fully understood, but studies on its origin and evolution may provide valuable informations.

A. niger is the one of the most important platforms in biotechnology, with the production of organic acids, proteins, enzymes and secondary metabolites. Aox has been shown to play a key role in citric acid fermentation: as an alternative respiratory pathway, it uncouples NADH regeneration from oxidative phosphorylation, thereby enhancing the glycolytic flux. Furthermore Aox requires increased aeration, which would lead to ROS formation, but the enzyme provides cell protection by eliminating them. A similar situation occurs during itaconic acid production by A. terreus, where stress conditions also increase the activity of Aox.

Recently, a study aimed at the evolution of aox paralogs in A. niger was undertaken in our laboratory. Using deletant mutant strains, the importance of both of the two aox genes present in the A. niger genome during citric acid fermentation was also demonstrated.

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Industrial Microbiology – Fungal Industrial Biotechnology, -

Production of probiotic microorganisms and examination of their cosmetic application

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The two most important lines of defense of the human body are the skin and the gastrointestinal tract. Probiotic microorganisms support the preservation of the latter's health in different ways, so the question arose, do they also have a beneficial effect on the skin?

In order to investigate this, we previously examined many representatives of the Lactobacillus genus and found positive effects in several of them, regarding skin moisture retention-, antioxidant- or even skin whitening effects [1].

Based on all of this, we included microorganisms from two commercially available preparations in our present study: Bacillus clausii and Saccharomyces boulardii. The strains of both species were produced by different fermentations in our lab, and then the resulting cells were revealed with ultrasonic desintegrator and examined with a dermatoscope to see if the skin moisture was higher or lower as a function of time, after cell extract was dropped on the skin of the tested persons (short-term hydration). Based on our results with Lactobacilli, a 4-12% increase can be expected regarding skin moisturizing effect of the probiotic cells.

[1] Tóth, P.; Németh, Á. Investigation and Characterisation of New Eco-Friendly Cosmetic Ingredients Based on Probiotic Bacteria Ferment Filtrates in Combination with Alginite Mineral. *Processes* **2022**, 10, 2672. https://doi.org/10.3390/pr1012267

Mycology, -

In Vivo Efficacy of Antifungal drugs against Candida auris Isolates

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Candida auris is a multidrug resistant and critical priority fungus against which echinocandins (anidulafungin, caspofungin and micafungin) are first line drugs. Rezafungin is the first new drug approved to treat candidemia and invasive candidiasis in more than 10 years. Rezafungin is a once-weekly, next-generation echinocandin with excellent in vitro and in vivo activity against the clinically important Candida species. However, data on in vivo efficacy of the four approved echinocandins against different C. auris clades are absent.

Ten isolates representing four C. auris clades (South Asian n=2; East Asian n=2; South African n=2; South American n=4; two of which were of environmental origin) were used. BALB/c male mice were given cyclophosphamide 4 days before infection (150 mg/kg), followed by administration of 100 mg/kg cyclophosphamide every third day until the end of the experiments. In the lethality (ten mice/group) and fungal tissue burden experiments (five mice/group) mice were infected intravenously (107 and 8x106 CFU/mouse, respectively). Twenty mg/kg dose of rezafungin on days 1, 3 and 6; once-daily treatment for 6 days with 3 mg/kg of caspofungin (Cancidas®), 5 mg/kg of micafungin (Mycamine®) and 5 mg/kg of anidulafungin (Eraxis®), were initiated 24 hours post-infection. These doses correspond to the currently used doses of the four echinocandins in clinical practice. After 21 days, survival rates were compared using the Kaplan-Meier logrank test. Fungal tissue burden (kidneys, hearts and brains) on day 7 were analysed with the Kruskal-Wallis test with Dunn's post-test. Histopathological examination on day 7 with haematoxylin-eosin and Periodic Acid Schiff was also performed (two mice/group).

Echinocandin MICs were not higher than the tentative MIC breakpoints suggested by the Centers for Disease Control and Prevention. Regardless of isolates and clades all echinocandin regimens improved survival (P values were from <0.001 to <0.0001, Fig. 1). At day 7 the survival rates for South Asian, East Asian, South African, and South American clades were 80-100%, 40-100%, 60-100% and 70-100%, respectively with all treatment arms.

In the fungal tissue burden experiments all echinocandins frequently produced >3-log mean fungal kidney and heart burden decreases some of which were not statistically significant (Fig. 2), Rezafungin, regardless of the clades, produced 3-5 and 2-4 log CFU decreases in the kidneys and hearts, respectively. In contrast, echinocandins did not inhibit fungal growth in the brain.

The histopathological examination showed that all echinocandins were effective in sterilization of kidneys, with the exception of mice infected with clinical isolate from the South American clade and treated with micafungin. Rezafungin- and to a lesser extent caspofungin- treated mice, regardless of clinical isolates and clades, did not show fungal cells in their hearts. Echinocandins treated mice regardless of the clades always showed medium and/or large foci of fungal cells in their brains.

Consistent with prior echinocandin in vitro data demonstrating activity against WT strains, this class was highly efficacious in vivo against C. auris. Rezafungin activity regardless of clades was comparable to or better than the previously approved three echinocandins.

Mycology, -

Strain specific transcriptome changes exerted by isavuconazole exposure in Candida auris

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The sudden emergence of potential multidrug- and pan-resistant Candida auris isolates, combined with limited therapeutic opportunities, poses remarkable global challenges in healthcare settings. Isavuconazole (ISA), either alone or in combination, exhibits promise as a salvage therapy for difficult-to-treat C. auris infections. Understanding the molecular mechanisms underlying ISA treatment is crucial for developing novel therapeutic recommendations. Therefore, we investigated the gene transcription profiles of non-wild type (non-WT) and wild type (WT) C. auris isolates from the South Asian clade following ISA exposure using total RNA sequencing. The non-WT isolate was classified according to the previously reported tentative epidemiological cut-off value of ≤ 1 mg/L. ISA treatment resulted in the up-regulation of 158 and 134 genes and the down-regulation of 119 and 96 genes in the non-WT and WT isolates, respectively, compared with control samples. In general, ISA-treated isolates showed increased transcription of the transcriptional factor UPC2, the drug transporter MDR1, vacuolar calcium-ATPase PMC1, and several ergosterol biosynthesis genes, leading to the accumulation of toxic sterols, increased plasma membrane permeability, and disruption of calcium homeostasis. The WT isolate showed pronounced enrichment of genes involved in sphingolipid biosynthesis, adhesion, and drug transport. These findings suggest that alterations in membrane lipid composition and modulation of drug efflux transporters are critical processes contributing to ISA susceptibility in clinical WT isolates.

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Mycology, -

Effect of the transcription factor Yap1 on the infectivity of Candida auris.

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The multidrug-resistant yeast Candida auris was first described in Japan in 2009 and since than it has been identified in several countries. C. auris can cause bloodstream, wound and other infections, including respiratory and urinary tract infections. C. auris is a healthcare associated pathogen with high mortality. Treatment options are limited due to antifungal resistance. It has strong resistance to azole antifungal drugs. It can also form biofilms that can colonize (without infection or any symptoms) the skin of patients, helping to spread to others.

Whole genome sequence (WGS) analysis suggests the near simultaneous and recent independent emergence of different clonal populations on 3 continents. WGS revealed that isolates clustered into distinct clades according to geographical regions. The clades were separated by thousands of single nucleotide polymorphisms, but within each clade the isolates were clonal.

One of the main reasons for the continued spread of Candida auris is several virulence factors that allow the fungus to adhere to the skin surface, for example. In addition, pathogens must cope with oxidative stress in the human body, and understanding this process is therefore key to understanding and preventing C. auris infections. Global gene expression changes in response to oxidative stress are regulated by several transcription factors, of which the Yap1 bZIP (basic leucine zipper) transcription factor plays a dominant role in Saccharomyces cerevisiae baker's yeast. In our experiments, we knocked out the ortholog of the Yap1 transcription factor from a strain of Candida auris. Examination of the mutant made it susceptible to several oxidative stress agents and at the same time reduced its infectivity. The mutant did not show a significantly different immune response or epithelial infectivity.

Mycology, -

Examination of cross-kingdom interaction between Candida auris and Pseudomonas aeruginosa: friends or foes?

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Fungi and bacteria simultaneous colonization of various body sites may result interkingdom polymicrobial infections, which are frequently observed in the oral cavity, gastrointestinal tract, and respiratory tract. The relationship between Candida albicans and Pseudomonas

aeruginosa involves physical and chemical interactions and can be classified as antagonistic. However, data derived from C. albicans cannot be extrapolated to non-albicans species such as C. auris. Here, we investigated the interaction between P. aeruginosa and C. auris to reveal the nature of this relationship at physiological and molecular level. Concerning planktonic co-culture based time-kill experiments, there was no significant changes in living fungal cell number at first 6 hours. Between 8- and 24-hours significant C. auris colony forming unit reduction could be observed with Pseudomonas co-culture compared to the cell numbers produced by C. auris growing alone. Regarding the adhesion and biofilm forming ability, the nature of interaction between C. auris and P. aeruginosa was evaluated by LIVE/DEAD BacLightTM viability assay and calcofluor-white based staining. P. aeruginosa cells significantly inhibited the initial adhesion and early biofilm forming ability of C. auris. In case of one-day-old preformed C. auris biofilms, P. aeruginosa cells caused significantly higher ratio of dead fungal cells following 8 hours co-culture. Regarding C. auris transcriptome changes, fungal isolate was grown in yeast extract–peptone–dextrose (YPD) medium for 18 hours at 30 °C, diluted to optical density (OD640) of 0.1 in 20 mL fresh YPD broth and incubated at 37 °C for 4 hours with shaking at 140 rpm. P. aeruginosa was diluted to an OD640 of approximately 0.05 in YPD medium containing C. auris cells in exponential growth phase. The C. auris-P. aeruginosa mixed cultures and C. auris monocultures were incubated for an additional 2 hours at 37 °C then samples were collected for RNA isolation and further RNA-seq based total transcriptome analysis. Our obtained results contribute to the better understanding of the complex interactions within P. aeruginosa and C. auris co-cultures.

Mycology, -

Aspergillus fumigatus has efficient protection against iron overload stress

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Microorganisms must successfully cope with iron limitation stress in order to survive in the human body. Iron efflux systems, such as Fe2+ ATPases, are important virulence factors for many human pathogenic bacteria. However, such systems have not been described for human pathogenic fungi. Here, we compared the transcriptome of FeSO4-stressed or unstressed iron-limited and iron-supplemented cultures of Aspergillus fumigatus. Iron limitation induced by deferiprone (DFP) treatment resulted in enrichment of siderophore cluster genes and reductive iron assimilation (RIA) genes in the upregulated gene set. Genes encoding Fe-S cluster proteins, heme peroxidases and catalases, and proteins of the tricarboxylic acid cycle, aerobic respiration, and ergosterol biosynthesis were also enriched in the upregulated gene set. FeSO4 treatment caused redox imbalance and reduced growth in both iron-supplemented and iron-limited cultures. This treatment downregulated RIA but not siderophore cluster genes in iron-supplemented cultures. Surprisingly, FeSO4 treatment further increased transcription of RIA and siderophore cluster genes in iron-limited (DFP pretreated) cultures. It is hypothesized that intracellular siderophores used for iron storage and accumulated during iron limitation may effectively protect cells from iron overload. Efficient and safe iron storage may be an economical alternative to iron efflux systems during iron overload stress.

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Mycology, -

The presence of a near-terminal double-stranded RNA element is associated with near-identical [D1,2] stwintrons integrated in different, unlinked genes in species of Xylariales

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Spliceosomal U2 introns are pervasive in nuclear transcriptomes but their persistence or amplification in most contemporary Eukaryotic lines remains a vexing issue. Continuous intronic sequences do not necessarily consist of one canonical U2 intron. Complex intronic sequences called [D1,2] stwintrons consist of nested U2 introns, where an internal intron splits the 5'-donor of an external intron between its first and second nucleotide (nt). Other types of stwintron exist, e.g., [D2,3], [D5,6] or [A2,3], the latter where an internal intron splits the 3'-acceptor of an external intron. Nevertheless, almost all stwintrons described to date are of the [D1,2] type suggesting unique means for their duplication. Sequence-similar [D1,2] stwintrons –sister stwintrons– are typically integrated at new intron positions, specific for one species or for one group of strains of one species. Recently, 285 sequence-similar [D1,2] stwintrons were cross-identified during coordinated blastn screens and retroscreens in the genomes of 14 Xylariales, using proven Hypoxylon sp. CO27-5 sister stwintrons as primary queries. Occasional missplicing was apparent during verification with matching RNA sequence reads from publicly accessible SRAs (NCBI), where almost the entire stwintron was excised by one splicing reaction. Clades of near-identical sister stwintrons were identified in Xylaria sp. MSU SB201401 and Xylaria longipes which differ only by one-to-five nt (> 95 % identity in about 185 nt-long stwintrons) albeit integrated in completely different genes. However, these seven apparently species-specific, near-identical stwintrons share near-terminal inverted repeat elements of 10-nt length we named 5'-NTIRE-10 and 3'-NTIRE-10, which are fully complementary as RNA due to the wobble basepairing options, G:U and U:G. This characteristic provides circumstantial evidence for the crucial involvement of an RNA intermediate during [D1,2] stwintron duplication. The topical 5'-NTIRE-10 sequence is 5'-GUAUAAAAAC (or one-nt variants thereof). Complementary NTIRE-10 partners were also present in three Hypoxylaceae we investigated, including in Hypoxylon sp. CO27-5/EC38 which are intriguingly the very same as found in the three X. longipes near-identical stwintrons. In two other species, fully complementary NTIRE-10 partners appear the result of compensatory point mutations at opposite ends of the intronic sequence. 5'- and 3- NTIRE-10 partners can form a near-terminal stem structure that brings in close proximity the terminal G's of the [D1,2] stwintron and of its alternative misspliced intron, possibly providing protection against rapid exonuclease degradation. The exact folding of the interior intron RNA appears irrelevant. Ten of the 21 stwintrons with fully complementary NTIRE-10's are present in Xylaria sp. MSU SB201401 implying that [D1,2] duplication has most recently occurred more frequently in this species. There are also 17 stwintrons with fully complementary NTIRE-9's in our set, eight of them in X. bambusicola and another four in MSU SB201401.

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Mycology, -

Transcriptomic changes in Candida albicans in response to Neosartorya fischeri antifungal protein 2 pressure

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As a consequence of the increasing number of drug-resistant strains and the limited number of effective drugs, there is an urgent need to develop new antifungal strategies to treat Candida infections. A small molecular weight, cationic and disulphide bridge-stabilized extracellular antifungal protein, the NFAP2, secreted by Neosartorya (Aspergillus) fischeri NRRL 181 is a promising candidate. It effectively inhibits the growth of planktonic and sessile biofilm cells of different Candida species both in vitro and in vivo; however, the antifungal mechanism remains unclear. A prompt cell membrane disruption is supposed if NFAP2 is applied at minimum inhibitory concentration (MIC). Under the MIC, NFAP2 is taken up by Candida albicans cells, it is localized intracellularly and has a long term antifungal effect by disturbing the cell functions. In the present study, we investigated the transcriptomic changes of C. albicans after treatment with sublethal concentration of NFAP2 to get insight into the long-term antifungal effect. Transcriptomic data indicated that mainly the expression of genes related to membrane transport, biosynthetic and metabolic processes are down-regulated; while genes related to stress responses to cell wall integrity and starvation, and (negative) regulation of filamentous growth are up-regulated. These data well support our previous observations regarding the antifungal effect of NFAP2 in C. albicans, viz. the slow growth in presence of NFAP2 and the inhibition of biofilm formation.

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Mycology, -

Impact of biotic and abiotic factors on the composition of soil fungal communities in primary and secondary tropical rainforests

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Tropical rainforests are among the most biodiverse ecosystems in the world, but we still have much less information on microbiological diversity than in temperate forests. Not only are many of the species unknown, but also the biotic and abiotic factors that influence the composition of fungal communities at landscape level in the Tropics. Also unknown is the impact of anthropogenic disturbance, such as deforestation, and the role of fungi during secondary succession of regenerating forests. In this study, I analyzed community data of tropical tree species and soil fungi in primary and secondary rainforests in Costa Rica and evaluated the possible role of abiotic factors influencing them. My data suggest a strong correlation between tree and fungal community composition in both primary and secondary rainforests. Forest age, edaphic variables and regional differences in climatic conditions all have significant effects on tree and fungal species richness and community composition. In addition, I found significant

differences in beta diversity values between primary and secondary rainforests at different stages of succession: in the early stages of succession, I observed greater differences in both tree and fungal communities among young forest plots than in older secondary and primary rainforests. The results suggest greater environmental heterogeneity and greater stochasticity of community composition in the early stages of succession in secondary forests, and some convergence of tree and fungal communities to competitive species able to survive on scarce resources under more stable environmental conditions in old forests.

Mycology, -

Aflatoxin B1 and sterigmatocystin production of Aspergillus flavus and A. creber on corn and rice starches mounted on glass beads

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The production of aflatoxin B1 (AFB1) and sterigmatocystin (ST) by AFB1-producing Aspergillus flavus Zt41 and ST-hyperproducer A. creber 2663 mould strains on corn and rice starches of high purity and nearly identical amylose-amylopectin composition as sole carbon and energy source was studied.

Experience has shown that toxin production reaches its maximum under aerobic, non-disturbed, dark, 27-35 °C, 90-98 water activity, solid phase conditions, and much higher toxin yields can be achieved on rice than on corn grit. For this study, as starches are powdery substrates, a special glass bead system was developed to model the conditions on the surface of grains. AFB1 production of A. flavus Zt41 was measured as 437.6±128.4 ng/g and 90.0±44.8 ng/g, while A. creber 2663 produced 72.8±0.0 µg/g and 26.8±11.6 µg/g ST on rice and corn starches, respectively, corresponding to 5- and 3-fold differences for AFB1 and ST production. Scanning electron microscopy indicated average starch particle sizes of 4.54±0.635 µm and 10.9±2.78 µm, corresponding surface area to volume ratios (1/µm) of 127 and 0.49 for rice and corn and starches, respectively. Thus, a 2.5-fold difference in particle size corresponded to an obviously larger, 259-fold difference in surface area. So the differences in the toxin production are possibly related to the different surface areas.

Mycology, -

Investigation of the role of transcription factors in the azole resistance of *Mucor lusitanicus*

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Some members of the order Mucorales, as opportunistic human pathogens, are capable of causing often fatal infection, called mucormycosis, in immunosuppressed patients. Treatment of this infection is complicated by the increasing resistance to some antifungal agents used in the clinic, including azoles. The mechanism of action of azoles is based on the inhibition of the 14-*α*-demethylase enzyme encoded by the *erg11* gene. Inhibition of this enzyme results in the accumulation of toxic sterols in the cell membrane. In addition to mutations or duplications in the *erg11* gene, azole resistance can also be caused by the action of active efflux pumps, known as multidrug resistance proteins (MDRs). The ABC and MFS transporters that trigger this phenomenon are embedded in the plasma membrane and transport antifungal drugs and other toxic substances outside the cell. In addition, the activity of genetic elements and transcription factors (PDREs, Zn_2Cys_6) that regulate the above-mentioned processes may also play a role in the development of azole resistance. Recent studies report that the genes encoding ergosterol biosynthesis and ABC transporters show coupled regulation in yeasts and filamentous fungi. These studies suggest that a highly complex system, the PDR network, involving active transporters and genes involved in ergosterol biosynthesis, as well as other genes involved in the stress response and regulators and transcription factors that affect their function, underlies the resistance of pathogenic fungi to azoles. Based on RNAseq analysis and homologous search results, several genes encoding putative transcription factors were found in the Mucor genome database. Among these genes, we first selected those that may encode factors carrying fungal-specific transcription domains that are typically reported in the literature as carriers of transcription factors involved in stress response or in the development of resistance to antifungal agents, namely the Zn(II)2Cys6 or zinc binuclear cluster [PF00172] and the 'fungal-specific transcription factor domain' [PF04082] or 'Fungal_trans' domain. Based on the bioinformatic data and qPCR results, for further work, the *tf106* gene (the encoded protein carrying both the zinc-binuclear cluster and the Fungal_trans domains) and the *tf821* gene (the encoded protein carrying the zinc-binuclear cluster and showing high homology with the Candida glabrata TAC1 ortholog and the Aspergillus fumigatus AtrR transcription factor) were chosen to generate deletion mutants. Our results so far suggest that the transcription factors investigated play a role in the regulation of azole resistance in *Mucor lusitanicus*.

Keywords: *Mucor lusitanicus*, azole resistance, transcription factors, pleiotropic regulation, PDR network

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Mycology, -

The effect of Pseudomonas aeruginosa secreted quorum-sensing molecule on Candida auris and Candida albicans physiological and transcriptional properties

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Bacterial-fungal co-infections and related microbial interactions are frequently observed in clinical practice and associated with worse patient outcomes especially in critically ill patients, yet few have been well characterized at molecular and/or physiological level. The main Pseudomonas quorum-sensing molecule 3-oxo-C12-homoserine lactone (HSL), which is a regulator of bacterial virulence factor production, inhibited hyphal development, biofilm formation and induced apoptosis in most Candida species. Here, we performed a detailed physiological and molecular characterization exerted by HSL on C. auris and compared them to C. albicans related data. To reveal the molecular events induced by HSL, a genome-wide transcriptome profiling was performed with C. albicans and C. auris culture following 100 µM and 200 µM HSL exposure using total transcriptome sequencing. The growth of C. auris and C. albicans was examined following 100 and 200 µM HSL treatment in YPD. Adding HSL to precultured Candida cells resulted in a significant growth inhibition starting at 2 hours postexposure. Regarding adhesion, HSL exposure exerted a concentration-independent effect on metabolic activity in C. auris, while concentration dependent activity was observed in case of C. albicans. In vivo virulence was significantly affected by HSL in case of C. auris but not for C. albicans. The HSL exposure –regardless applied concentrations– resulted 44 (i.e.: regulation of cell-cell adhesion, arginine metabolic process) and 76 (i.e.: mitotic cell cycle, chromosome localization, microtubule-based process) genes with at least 1.5-fold increase or decrease in transcription for C. albicans, while 45 (i.e.: fatty acid catabolic process, peroxisome) and 25 genes was observed with at least 1.5-fold increase or decrease in transcription for C. auris. The described differences on adhesion, biofilm forming ability, virulence and intracellular metal milieu with global transcriptome analysis suggest that HSL based interaction may have a significant impact in C. auris-P. aeruginosa relationship compared to C. albicans-P. aeruginosa co-occurrence.

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Mycology, -

Establishment of filamentous Candida auris cells in liquid culture to investigate the antifungal mechanism of an antifungal protein

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Candida auris is one of the most hard-to-treat human pathogenic fungus due to its high virulence and resistance to conventional antifungal drugs. As a virulence factor, C. auris is able to form biofilms on the skin and the surface of medical instruments which allow its easy and fast spreading in hospital environment and causing nosocomial infections. In the biofilm, the planktonic C. auris yeast cells are aggregated or grow in pseudohyphal or hyphal forms. The ability to form these cell types varies between the strains. The clinical C. auris isolates are usually able to form filamentous cells after passage through a host. The emergence and spread of multi- and pan-resistant C. auris strains make it urgent to develop novel antifungal compounds with different mode of action than that of the conventional ones. In our previous studies, we demonstrated that Neosartorya (Aspergillus) fischeri NRRL181 is able to produce a small molecular weight, cationic, cysteine-rich, highly stable protein with high anti-Candida activity, namely the N. (A) fischeri antifungal protein 2 (NFAP2). Sessile biofilm cells of C. auris proved to be susceptible to NFAP2 both in vivo and in vitro, which makes NFAP2 a promising antifungal compound to inhibit and eradicate C. auris biofilms. The first step of this goal is the understanding the biofilm inhibitory and eradication mechanisms of NFAP2 which requires easy-to-handle filamentous C. auris cells. In the present study we aimed to develop a method to grow C. auris cells in filamentous form in liquid culture. A low nutrient culture medium supplemented with 2,5% NaCl proved to be appropriate to grow C. auris NCPF 8971 cells in filamentous form without application of strong genotoxic stressors. After 2 hours of transferring filamentous cells from this medium into a NaCl-free one, the filamentous cells reverted into planktonic. It indicated that the high salt concentration facilitated the filamentous growth of this strain. Filamentous C. auris NCPF 8971 cells proved to be susceptible to NFAP2 with minimum inhibitory concentration at 50 µg/ml, which killed all cells within 2 hours. Experiments with fluorescent BODIPY-labelled NFAP2 indicated that it is localized in outer layers of filamentous C. auris NCPF 8971 cells after the treatment. These results allow us to conduct reliable transcriptomic studies in the future to get insight in the antifungal mechanisms of NFAP2 in filamentous C. auris cells.

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Mycology, -

Evolutionary Dead Weight Reduction: Sacrificing Redundant Functions for a Pathogenic Lifestyle

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During evolution, fungi have explored various lifestyles. Each of these different lifestyles required distinct gene sets. Pathogenicity is one such lifestyle, which entails that the significance of some genes and functions increases, while others become redundant. It has also been observed that pathogenic lifestyles have independently evolved in phylogenetically distant species and cannot be traced back to a single ancestral pathogenic species. It is possible that certain functions may become advantageous, leading to the duplication of associated genes. This can result in the development of fine-tuning regulation, and alternative functions may also emerge to aid in adaptation to the new lifestyle. We also know such cases where a function becomes evolutionarily redundant and it disappears from the genome. Based on all of this, it is conceivable that there may be common gene gains or losses characteristic of species with a pathogenic lifestyle, which cannot be traced back to a single common ancestral fungal species, but rather occurred independently and in parallel across different phylogenetic taxa.

So the main questions of our research were: (1) Are there particular gene gains that are common and crucial for the adaptation to a pathogenic lifestyle? (2) Is there a specific function loss that is initially targeted for removal when a fungus transitions to a pathogenic lifestyle?

For this study, first we selected representative species from each group from insect pathogens (Beauveria bassiana), nematode pathogens (Drechmeria coniospora), obligate human pathogens (Blastomyces dermatitidis), and facultative human pathogens (Aspergillus fumigatus) to determine which genes they have gained or lost and whether there are common elements among them. To observe gene gain or loss events that are not merely phylogenetically traceable, we chose control species that are more closely related to the pathogenic species than the pathogens are to each other. This way, any observed gene gains or loss events had to occur independently and in parallel.

We found that there are gene duplications that occur in the pathogenic species and it does not appear in the control species. However, it does not characterize the pathogenic lifestyle in general, because it is rather only specific to a given pathogenic species.

We found that only one specific function – plant cell wall degradation – was lost, which had originally evolved in the Pezizomycotina taxon to support a saprophytic lifestyle. Although others have also reported that cell wall degradation genes disappear in some pathogenic fungi, we have confirmed that this is the only characteristic sacrificed by fungi that have transitioned to a pathogenic lifestyle. We collected and examined all sequenced fungal species characterized as either insect or human pathogens, as well as phylogenetically close saprophytic fungi, to validate this statement. We also demonstrated that obligate and facultative pathogens differ in this trait. We identified marker genes that are likely to be lost in obligate pathogenic fungi but are present in facultative pathogens, plant pathogens, and saprophytic species. These marker genes may can help determine whether a sequenced Pezizomycotina fungal species is an obligate pathogen.

Mycology, -

Untargeted metabolomic analysis of *Alternaria* sect. *Alternaria* in grapevine

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The *Alternaria* genus is a dominant member of the grapevine (*Vitis vinifera*) mycobiome. Numerous *Alternaria* species are known for producing various secondary metabolites that are key in plant protection and food safety. Using single-locus and multi-locus phylogenetic analyses, we identified two distinct *Alternaria* groups in grapevine: *A. alternata* and the *A. arborescens* species complex (AASC). Given that the analysis of secondary metabolite production is a widely used chemotaxonomic method, we examined whether the untargeted metabolomic analysis of *Alternaria* isolates derived from grapevine supports the separation of these two phylogenetic groups.

In this study, we identified the secondary metabolites produced by *Alternaria* isolates and determined their metabolite profiles using liquid chromatography coupled with mass spectrometry (LC-MS). The untargeted MS data processing revealed the presence of 647 and 453 metabolites in positive and negative ionization modes, respectively. Using a PLS-DA model, we demonstrated that the metabolic data for *A. alternata* and AASC are distinct. Through univariate analysis based on the discriminatory power of the metabolites, we identified metabolic compounds (altenuene, L-tenuazonic acid) that showed significant quantitative differences between *A. alternata* and AASC. This reinforces the separation between *A. alternata* and AASC, which had been previously uncovered and is now further supported by our molecular phylogenetic analysis.

Mycology, -

bZIP-type transcription factors in Aspergillus flavus and Fusarium verticillioides

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Aspergillus flavus is a saprotrophic and pathogenic fungus, which is responsible for severe agricultural damage to crops, often causes fatal aspergillosis in humans, and induces liver damage and immunosuppression in mammals through the production of the secondary metabolite aflatoxin. Fusarium verticillioides is primarily known for its agricultural damage, mainly in forage plants and it also requires special attention as a result of its fumonisin mycotoxin production.

In filamentous fungi bZIP type transcription factors coordinate stress response and production of secondary metabolites like mycotoxins. In this work we studied bZIP transcription factors in A. flavus and F. verticillioides. For this purpose, we created gene deletion and complementation mutants, verified by PCR tests. The wild type strains and the mutants were subjected to phenotype tests, including oxidative as well as osmotic, cell wall integrity and heavy metal stress sensitivity studies. We also performed quantitative determination of mycotoxin production of the strains. Based on our results, the deletion mutants show the expected sensitivity to oxidative stress, in some mutants we observed alteration in the mycotoxin production, while the complemented and the wild type strains show similar phenotype.

In the following we will carry on RNA sequencing to identify the genes regulated by the transcription factors and we will also start corn seed infection experiments to determine the virulence of the mutants.

Our basic research can be exploited for agriculture, drug development and food industry.

Mycology, -

A Compendium of Saccharomyces yeasts with sequenced genomes

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Knowing and understanding the strains behind sequenced genomes is crucial to fully exploit microbial population genomic advancements. By a comprehensive evaluation of more than 300 publications, we compiled a curated list, a Compendium of ~5,400 Saccharomyces short-read genomes, the strains they represent, and their metadata. We gathered information on the isolation date and geographical location of the isolations.

We created a new, comprehensive ontology of the isolation sources, and for commercial strains, we collected data on the manufacturing companies and the type of formulation. We collected equivalent strain names and indicated equivalent genome sequences. Approximately 80% of the collected strains are Saccharomyces cerevisiae and 3% are hybrids. Isolation sources overwhelmingly fall into the categories of 'Agriculture and Food' (57%), ‘Nature’ (28%), and ‘Human’ (11%). About 26% of the isolates come from fruit fermentation and 22% from grain fermentation. One-third of the yeast genomes are European, with 10-20% being Asian, North American, and Oceanian isolates, and the remaining 5-5% are from Africa and South America. In this study, we aimed to take a synthetic approach to both large- and small-scale population genomic and phylogenomic studies to define clades and mosaic groups by analyzing all available sequenced Saccharomyces genomes. Focusing on S. cerevisiae, we show how all the clades ever described in Saccharomyces literature compare to each other and how phylogeny is affected when only individual chromosomes are considered. Furthermore, by identifying single-spore isolates and non-sporulated equivalent strains, we show that phylogenomic placement is often substantially affected when spore clones are used. The Compendium is accompanied by a collection of gvcf files, allele ratio plots, and coverage

plots for all genomes, providing the opportunity for large-scale genomic analyses with historic, biogeographic, and ecologic context.

Our goal is to create and maintain an online, continuously updated database accessible to everyone, containing strain and genomic data of short-read sequenced Saccharomyces, later amended with long-read sequences and assemblies. The non-exhaustive list of relevant literature on each sequenced strain aids in understanding the organism behind the sequence data. Finally, we also highlighted regions and sources of yeasts underrepresented and understudied.

Mycology, -

Characterisation of evolved fluconazole resistant Cryptococcus neoformans strains

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Cryptococcus neoformans, an opportunistic pathogenic yeast in humans, is responsible for 100% fatal meningitis in individuals lacking requisite T-cell functionality, which may also endanger immunocompetent hosts by infecting the respiratory apparatus (1). This organism is recognized for virulence factors, including the ability to grow at 37 °C, the production of a polysaccharide capsule and melanin (2).

Until 2017, treatment of cryptococcosis predominantly relied on amphotericin B and 5-fluorocytosine. Subsequently, a third antifungal agent, fluconazole, has been incorporated into treatment regimens. However, due to the high prevalence of primary resistance of 5'-fluorocytosine and the occurrence of fluconazole resistant strains complicates the therapy (3). The emergence of resistant strains can be attributed to the superfamily of ABC-transporters, which play a crucial role in the transfer of antifungal agents from the intracellular space. ABC-transporters encompass several subfamilies that are classified based on their structural characteristics. The functional attributes of homologous transporters present in diverse fungal strains and mammalian species have been extensively examined. PDR-type transporters, characterized by one transmembrane domain and one ATP-binding domain, are categorized within the ABCG subfamily, which is specifically conserved in pathogenic organisms and predominantly mediates antifungal resistance, being exclusive to plants and fungi.

Fluconazole-resistant mutant strains of C. neoformans were generated through the in vitro evolution of the C. neoformans IFM 5844 strain in the presence of fluconazole; the strains named R1 type exhibited a dark brown phenotype, while the R2 strains displayed a pale brown coloration. The melanin synthesis of both the parental, R1 and R2 type resistant strains was analyzed via HPLC to elucidate the basis for the observed coloration differences and RT-PCR was conducted to investigate the expression levels of the laccase 1 gene (CNLAC1). The expression of YCF1 homologoue transporter gene (CNA08000) was also examined to reveal the role of this transporter in the resistance.

Notwithstanding the brown pigmentation of the resistant strains, melanin could not be identified within these organisms. The RT-PCR results indicated that the CNLAC1 gene is not expressed in the strains exposed to fluconazole. The expression of the YCF1 homologue transporter gene is significantly induced in the presence of fluconazole, exhibiting two-fold increase in expression rate in the R1 resistant strain relative to the parental strain.

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Mycology, -

Forestry practices and interannual variation shape ectomycorrhizal fungal community composition in an oak-hornbeam forest in northern Hungary

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The purpose of this study was to better understand the effects of forestry treatments (clear-cutting, gap-cutting, preparation-cutting, tree retention in clear-cut areas, and control) on the diversity and community compositional of ectomycorrhizal fungi an oak-hornbeam forest in northern Hungary. We sampled soil samples in all 30 plots of the Pilis Forestry Systems Experiment, operated by the Ecological Research Centre and the Pilis Park Forest Ltd., in the autumn of 2020 and 2021. We performed ITS2 rDNA metabarcoding of the soil-borne fungal communities, completed bioinformatic analyses, and assigned the resulting high-quality fungal sequences to taxonomic and functional groups. For this presentation, we compared the richness and community composition of ECM fungi among forest treatment types and between years using analysis of variance for alpha diversity and nonmetrical multidimensional scaling and permutational analysis of variance for beta diversity analyses. Out of the 1035 detected fungal genotypes, 267 represented ECM fungi, of which the five most diverse phylogenetic clades were /tomentella-thelephora (47), /inocybe (40 genotypes), /russula-lactarius (52), /sebacina (27), and /cortinarius (20). We found significant variations in ECM fungal richness among forest treatment types, showing similar patterns on both year. Specifically, clear-cutting plots had significantly lower richness of ECM fungi than control, preparation cutting, and retention tree groups. This was particularly evident in /amanita, /cenococcum, /russula-lactarius, and to a smaller extent in /tomentella-thelephora, while /inocybe did not show strong treatment effect. We did not find significant differences in richness between the years. Conversely, there was a significant compositional difference between the two years, in addition to the strong treatment effect. Treatment was partly explained by altered environmental variables, such as soil water content, microclimate (predominently relative humidity and upper soil temperature), understory vegetation, and distance from the plot to surrounding ECM host trees, while interannual variation may partly be caused by differing weather conditions during growing season, as the year 2021 was noticeably drier than 2020.

Mycology, -

Heterologous expression and identification of a Solanum lycopersicum L. antifungal defensin-like protein

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The enormous amount of crop losses caused by phytopathogenic fungi is a serious problem in agriculture due to the fast spread of fungicide-resistant strains. Therefore, new antifungal strategies are required. Plant defensins are promising biofungicide candidates due to their broad antifungal activity and environmental stability. In the present study a novel defensin-like protein (B1N680) from Solanum lycopersicum L. was produced extracellularly in a Pichia pastoris-based expression system, which allowed the bulk production (~8.5 mg/l). According to the result of electrospray ionization mass spectrometry (ESI-MS) analysis, a glutamine-alanine (EA) amino acid residues remained at the N-terminus of the recombinant B1N680 due to the incorrect cleavage of the extracellular alpha-factor singal sequence. Furthermore, ESI-MS data indicated the presence of four disulphide bridges between eight cysteine residues. The structure of B1N680 was investigated with electronic circular dichroism spectroscopy and reversed-phase high-performance liquid chromatography. These indicated folded, disulphide bridge stabilized structure of B1N680 constituted by α-helix and β-sheet. The antifungal activity and stability of B1N680 was determined in in vitro microdilution susceptibility tests. It was observed that B1N680 has fungicide growth inhibitory effect at minimum inhibitory concentration range of 6.25 µg/ml – 25 µg/ml against several plant pathogenic filamentous fungi (viz. Botrytis cinerea, Cladosporium herbarum, Trichoderma harzianum, Fusarium spp.,), and it is fungistatic against Aspergillus spp. Furthermore, it was demonstrated that, the B1N680 preserved its antifungal activity after heat (50 °C, 100 °C for 60 minutes) treatment and ultraviolet irradiation against C. herbarum, although the slightly acid and basic pH decreased its efficiency. Based on these results, B1N680 is a promising biofungicide molecule, however several plant toxicity and protection experiments are required to prove it.

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Mycology, -

CotH proteins are necessary for normal hyphae and spore formation and virulence in Mucor lusitanicus

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Understanding the pathomechanism of mucormycosis, host-pathogen interactions with Mucorales fungi, and identifying virulence factors are critical. Our research focuses on the detailed analysis of spore coat (CotH) kinases and other surface proteins through phenotypic

examination of CRISPR-Cas9-derived mutants. Disruption of specific cotH genes led to alterations in spore coat structure, spore size distribution, fungal growth, sporulation, septa formation, overall fungal survival, and interactions with macrophages. Importantly, we discovered that CotH proteins originated from bacteria via horizontal gene transfer, and their presence is not universal among all fungi.

CotH-mediated interactions are essential for fungal invasion, with a significant increase in GRP78 expression in sinuses, lungs, and brain during diabetic ketoacidosis (DKA), increasing susceptibility to Rhizopus oryzae infection. IgG antibodies targeting Rhizopus CotH3 conferred protection against mucormycosis in DKA mouse models, highlighting the therapeutic potential of anti-CotH3 antibodies for human immunotherapy. Viability assays revealed that deletion of cotH3, cotH4, cotH9, cotH12, and cotH13 genes significantly attenuated the pathogenicity of Mucor lusitanicus during in vivo Drosophila melanogaster infections. Interestingly, the cotH3 mutant showed reduced virulence in DKA mice with elevated GRP78 expression, but not in Galleria mellonella, despite lacking the characteristic motif identified in Rhizopus delemar.

Given its sequence similarity to Rhizopus CotH3 and the presence of the "CotH-motif," CotH4 may also act as a GRP78 ligand, warranting further investigation. Our findings suggest that CotH proteins play a critical role not only in the pathogenicity of M. lusitanicus, but also in spore size, structure, and host immune interactions, reflecting their evolutionary significance following bacterial horizontal gene transfer. Importantly, based on their surface localization and antigenic properties, CotH3 and CotH4 were identified as surface-exposed proteins.

Mycology, -

Mead yeasts: a student research project at the University of Debrecen

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Mead is an ancient beverage produced by diluting honey with water and subsequent fermentation with yeast. It has a rich but almost-forgotten tradition throughout the world and probably has been produced and consumed since paleolithic times. Its popularity has declined since the Middle Ages and only a handful of traditional styles remained. A revival of this drink and a proliferation of craft meaderies started in the USA in the 1980s, a trend that has continued throughout the years, similarly to the story of craft beer. Meadmakers originally applied Champagne and other wine yeasts. In the past few years, yeast manufacturers stepped into the niche market of meadmaking supplies and begun distributing dedicated mead yeasts. The origins of these strains in unknown. On a much larger scale, craft and homebrewing has also seen a proliferation in available yeast strains.

At the University of Debrecen, microbiological laboratory courses for the recently started Biotechnology BSc programme were designed to introduce students to microbiological and biotechnological topics in a way that enables them to participate in an ongoing research project. Two such projects have been conducted, the first focusing on craft and traditional beer bottle isolates and their genetic comparison, the second revolves around mead yeast. For the

mead project, we obtained mead yeast product from the US, UK, Sweden, Poland, and Czechia. Isolates from all products were subjected to various tests. All isolates tolerated high osmotic stress (up to 40–50 w/v % glucose). Killer activity was variable. Fingerprinting identified a group of 5 products most likely containing the same strain, however, the individual isolates differed in killer activity, highlighting isolate-level variations. Next, dry meads were prepared in 1 L fermentations with all mead yeasts in triplicates. After a 6-week fermentation at 18°C, general characteristics of the produced meads were measured (alcohol content, final gravity, titratable acidity, volatile acids, etc.) along with aroma components using the HS-GC-MS method. Participants of the class were asked to perform sensory evaluation (bouquet and flavor) and rating of the meads. Analytic and sensory data were compared and discussed with students. Students who successfully completed the course and took part in interpreting the results were included in a manuscript on mead yeasts as group authors, thereby getting insight into research and the scientific publishing process. Our observation is that student engagement was positively affected by merging education and research. In addition to the aforementioned experiments, mead yeasts' genomes were also sequenced, showing that they were members of the wine, mixed origin, or Asian clades.

Mycology, -

Does a colony of Aspergillus nidulans respond to sterigmatocystin produced by neighboring colonies?

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Stress usually alters the spectrum of secondary metabolites of fungi. Detecting these changes may inform the colony about the physiological status of neighboring colonies and may help it to prepare for upcoming changes in the environment. Here, we tested this hypothesis by evaluation of genome-wide transcriptional changes induced by the addition of sterigmatocystin to Aspergillus nidulans cultures grown under conditions unsuitable for the production of this metabolite. Sterigmatocystin upregulated 514 genes and downregulated 894 genes, although it had no effect on growth. The upregulated gene set was enriched in genes for rhamnogalacturonan degradation or extracellular proteases, which usually show high transcriptional activity in carbon stressed, sterigmatocystin producing cultures. The xprG transcription factor gene regulating carbon stress response was also among the upregulated genes. The downregulated gene set was enriched in genes for glycolysis, the citric acid cycle, ergosterol biosynthesis and antioxidant enzymes, which generally show high transcriptional activity under fast growth conditions when cells do not produce sterigmatocystin. In general, the transcriptome changes induced by sterigmatocystin in the presence of glucose showed a positive correlation with the changes recorded under carbon limitation or carbon deprivation stress. Despite of it, no significant differences were observed in glucose utilization of the cultures, and after glucose depletion, in the development of carbon starvation stress markers (decrease in dry cell mass, hyphal fragmentation, extracellular γ-glutamyl transferase and β-glucosidase secretion). However, conidiophores developed earlier in sterigmatocystin treated cultures. Based on these results, sterigmatocystin treatment alone does not induce the carbon starvation stress response, nor does it increase the intensity of the carbon starvation stress response, but it may contribute to the sensing of this stress.

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Mycology, -

Vonalkód (amplikon) szekvenálás, genomszekvenálás: bonyodalmak a gombataxonómiában.

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A modern, molekuláris taxonómia a vonalkód-szekvenciák összehasonlítása alapján definiál taxonómiai egységeket és határozza meg a törzsek (izolátumok) taxonómiai hovatartozását. A vonalkódok olyan DNS szakaszok a genomban, amelyek megfelelői minden szervezetben megtalálhatók, és szekvenciáik viszonylag lassan változnak az evolúció során. Elsődleges vonalkódoknak szokás nevezni az rRNS cistron nem-kódoló ITS régióját, valamint az LSU és az SSU riboszómális RNS-eket kódoló gének bizonyos régióit. A másodlagos vonalkódok konzervált fehérjéket (pl. RPB1, RPB2, ACT1, TEF1, stb.) kódoló gének egyes szakaszai. A két kategória együtt is csak egy rendkívül kis hányadát jelenti a szervezet genetikai állományának. A rájuk épülő evolúciós, filogenetikai és taxonómia elemzések nem vehetik figyelembe a genom más részeiben rejlő filogenetikai információt. A genomszekvenálási technika lehetőséget nyújt a vonalkódokon kívüli genomrészek információ-tartalmának az elemzésére is. Az egyik irány bevonja az analízisbe az úgynevezett háztartási géneket. A másik módszer nagyszámú további ortológ gént (illetve génterméket) vizsgál. További lehetőség a teljes genomszekvenciák (nem csak gének) összehasonlítása. Mindegyik megközelítésnek vannak előnyei és korlátai is. Bonyodalmakat okozhat az rRNS cistronok homogenizálatlansága (többféle vonalkód-szekvencia jelenléte a genomban), genomon belüli heterozigótaság, alloploidia (hibridizálódás), alloaneuploídia, genom-kimerizálódás (a genom eltérő fejlődéstörténetű mozaikokból áll), horizontális géntranszfer, haploid genomszekvenciák összerakása (haploid assembly) heterozigóta genomokból (haploid konszenzus-szekvencia), stb. A felsoroltak korlátozhatják az elemzésekből levont következtetések megbízhatóságát a taxonómiában valamint a metabárkódozásos és metagenomikai diverzitás-vizsgálatoknál.

Mycology, -

Investigation of the phytase enzyme activity of saprobiontic fungal communities from the benthos of Kis-Balaton water protection system

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The microorganisms found in sludge play a fundamental role in the transformation and degradation of suspended solids and organic matter in surface waters, and in particular in the nitrogen and phosphorus cycles. This contributes to the stability of aquatic ecosystems. In a previous study, we conducted a quantitative culture study of the fungal community of the benthos saprobiont and compared the diversity of fungal communities. Subsequently, 1-1 L samples were obtained from the upper 10 cm layer of the mud at three points (A, B, C) along

the internal water flow of Kis-Balaton Water Protection System using an AMS discrete stratigraphic sampling device. The quantitative culture analysis of the samples on Bengal rose agar at 25°C at sampling points A, B and C revealed fungal abundances of $8.52x10^4$ CFU/g dry matter, $3.45x10^5$ CFU/g dry matter and $4.74x10^4$ CFU/g dry matter, respectively. A total of 383 random isolates were subjected to morphological characterisation, resulting in the differentation of 17 distinct morphotypes. The representative strains of each morphotypes were identified through the analysis of the nucleotide sequence of the ribosomal RNA ITS1 and ITS2 genes. The identification of 129 isolates from the sludge cultivated in the nearest Zala river inlet, sampling site A, permitted the isolation of 13 species, with Aspergillus jensenii and Cladosporium cladosporoides being the most abundant. The identification of 116 isolates from sampling point B, representing the middle section between inlet and outlet, yielded 9 species, with the most common being Cladosporium sphaerospermum and Neosarcochyta graminicola. The molecular identification of the representatives of 138 random isolates from the longest submerged sampling point C in Kis-Balaton yielded 8 species belonging to the order Pleosporales. Phytase enzyme activity of the strains was expressed by the quantity of orto-phosphate ions released from phytic acid.

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Mycology, -

Anticryptococcal activity of plant antimicrobial peptide derivatives

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The saprophytic, basidiomycetous yeast Cryptococcus neoformans exhibiting a worldwide distribution, possesses the capacity to provoke life-threatening meningitis in individuals with compromised immune systems, notably those afflicted with AIDS. Due to the high incidence and severe consequences of the infection, alongside a restricted number of therapeutic agents, cryptococcosis represents a substantial global health concern (1).

The standard treatment regimen for cryptococcosis involves the administration of amphotericin B in conjunction with 5'-fluorocytosine for severe cases. Recently, fluconazole has emerged as a recommended alternative, demonstrating comparable efficacy. The emergence of fluconazole and flucytosine-resistant strains, in addition to the potential for severe adverse effects associated with prolonged amphotericin B usage, underscores the necessity for the investigation of novel therapeutic strategies (2). Antimicrobial peptides are regarded as promising candidates, given their antifungal properties and minimal cytotoxic effects (3).

In this study, the anti-cryptococcal efficacy of Medicago truncatula NCR247, NCR335, and NCR169C derivatives, as well as three synthetic chimera peptides, was assessed. Sixteen peptide derivatives, along with all three chimeras, demonstrated notable effectiveness against Cryptococcus neoformans IFM5844 strain. Recombinant peptides proved the most potent ones having lower MIC than amphotericin B. Among these, the X1-NCR247C chimera exhibited the highest efficacy, with its effectiveness being contingent on both concentration and

time. The role of the cell wall, in anchoring the peptides to the cell surface is highlighted. Moreover, the peptides' influence on murine macrophages was also examined, showing their low cytotoxicity. Notably, it was demonstrated that the attachment of X1-NCR247C to yeast cells augmented their uptake by murine macrophages.

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Mycology, -

Mucoromycota tannases: activity screening, production and enzyme protein purification

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Tannases belong to the family of esterases and catalyze the degradation of hydrolysable and complex tannins to gallic acid and glucose. These enzymes are industrially important, as they are widely used as clarifying agents in beverage preparations and to improve the nutritional composition of feed products. Microbial tannases can be produced on tannin-containing agricultural waste materials. To improve the enzyme yield, there is a need for optimized solid-state or submerged production systems based on plant residue substrates, as well as for potentially active microorganisms applicable in fermentations. The cost of tannase production can be significantly diminished with such innovative applications. Microbial tannases have been described from fungi, mainly from Penicillium and Aspergillus isolates. Members of Mucoromycota, however, are less studied in terms of the tannase production. In this work, an attempt was made to screen Mucoromycota isolates for their tannase activity, including analysis and optimization of enzyme production under submerged (SmF) and solid-state fermentation (SSF) conditions. Isolation and characterization of tannase enzymes from the producer strains were also planned in this pilot project. Screening for tannase production was performed in plate tests using a growth medium supplemented with tannic acid as an enzyme production inducer. Isolates of the Rhizomucor miehei, Mucor corticolus, Mucor lusitanicus, Rhizopus microsporus var. oligosporus and Rhizopus oryzae species showed tolerance against the presence of the inducer, and tannase production was also detected in these strains after incubation. SSF systems contained wheat bran and grape pomace as substrates, and the extraction efficiency was studied by various extraction buffers. SmF assays were conducted under tannic acid supplemented yeast-peptone environment. Tannic acid proved to

be an excellent inducer of tannase production, and the wheat bran-based SSF system supplemented with tannic acid resulted in the highest enzyme yields. In SSF tests, the R. microsporus var. oligosporus and M. corticolus demonstrated the highest tannase activity, while the R. miehei was an excellent tannase source in SmF condition. Tris buffer extraction followed by anion exchange chromatography resulted tannase active protein fractions from M. corticolus. Optimum temperature condition for the isolated tannase activity was 30 °C. Our results highlighted Mucoromycota fungi as potential tannase producers for future investigations; as we know, the M. corticolus tannase activity is the first Mucoromycota tannase that has been purified. This research was supported by the projects NKFI FK 134886, HUN-REN 2001007 and TKP2021-EGA-28.

Mycology, -

In Vitro and In Vivo Efficacy of Echinocandins Against Iranian (Fifth Clade) Candida auris Isolates

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For the currently classified six clades of C. auris isolates, data on the in vitro fungicidal activity and in vivo efficacy of antifungal agents are only available for the first four clades. In our work, we determined the in vitro killing activities of anidulafungin, caspofungin, micafungin, and rezafungin using time-kill curves against five clinical isolates belonging to the fifth clade. In a neutropenic mouse model, mice intravenously infected with the MRL40 and TML617 isolates were treated intraperitoneally for six days with humanized echinocandin doses (where the area under the curve for the dose is equivalent). At concentrations of 0.5-32 mg/L, the four echinocandins only against the MRL40 isolate exhibited fungicidal activity. In the cases of the remaining four strains, only fungistatic effects were observed; regrowth was always noted for the isolates IFRC2087 and IFRC4050. For the TML616 and TML617 strains at ≥0.25 mg/L of anidulafungin and micafungin consistently resulted in positive killing rate values. All four echinocandins were effective in the lethality experiments against the MRL40 isolate (P<0.0002 for all four echinocandins), while for TML617, only rezafungin significantly increased survival (P<0.0049). In tissue persistence studies, the four echinocandins resulted in at least a four-log CFU reduction in the heart and kidneys for the MRL40 isolate, but in the brain, the number of cultured fungal cells was always at least 10^5 CFU/gram. In mice infected with the TML617 isolate, CFU reduction was significant except for anidulafungin, with only rezafungin and caspofungin being effective in the heart, while no echinocandin was effective in the brain. Based on our results, the in vitro and in vivo efficacy of echinocandins against Iranian C. auris isolates is echinocandin-specific and isolate-dependent.

Mycology, -

The nitrosative stress response elicited by nitrite depends on the MeaB bZIP transcription factor in Aspergillus fumigatus

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MeaB is a bZIP-type transcription factor in the human pathogenic fungus Aspergillus fumigatus, involved in maintaining cell wall integrity and contributing to the fungus's virulence. Given that MeaB has been described as a regulator of nitrogen metabolism in Aspergillus nidulans, we investigated its function in nitrite utilization and /or detoxification. We developed a meaB deletion mutant from the Af293 wild-type strain via electroporation and analyzed it using a transcriptomic approach to better understand its increased sensitivity to NaNO2.

In the wild-type strain, $NaNO_2$ treatment caused redox imbalance and inhibited growth. This growth inhibition was more pronounced at acidic pH, where NO formation from nitrite is increased. Genes associated with nitrosative stress (fhpA, fhpB, gnoA) and nitrate assimilation (niaD, niiA) were upregulated. The co-regulation of nitrate assimilation (niaD, niiA) and flavohemoglobin NO dioxygenase (fhpA) gene activities may prevent the accumulation of reactive nitrogen intermediates (RNI) during nitrite utilization as a nitrogen source. This regulation likely helps convert nitrite and/or RNI into less harmful ammonia under nitrosative stress. Additionally, genes involved in iron uptake, siderophore metabolism, and the iron-limitation stress response transcription factor hapX were upregulated. This response may help re-synthesize metalloproteins inactivated by nitrosative stress and facilitate the safe storage of iron. The upregulation of the amcA gene is particularly notable, as the encoded mitochondrial ornithine transporter may link iron metabolism, siderophore synthesis, and arginine-dependent NO production. Under nitrosative stress, this regulation may shift ornithine toward siderophore production, thereby reducing arginine-dependent NO production.

Deletion of the meaB gene increased sensitivity to nitrite and menadione sodium bisulfite (MSB), reduced conidiation on arginine as a sole carbon source, upregulated siderophore biosynthesis genes, and altered the transcription of arginine metabolism genes. We hypothesize that these changes result from disrupted arginine-dependent, endogenous NO production.

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Mycology, -

In vitro antifungal activity of two potentially Fe-S cluster protein inhibitors on Aspergillus fumigatus

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Virology, -

HIV-1 recombinant forms identified by near full-length genome sequencing in Hungary

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The high genetic variability of human immunodeficiency virus (HIV) leads to the rapid genomic diversification of the HIV epidemic, which may affect prevention, diagnostic and treatment efforts.

Motivated by the increasing proportion of HIV-1 recombinant forms worldwide and in Hungary, the aim of our study was to identify and characterize unique and circulating recombinant forms by near full-length genome (NFLG) sequencing for the first time in our country.

Our starting data were generated in the frame of routine HIV-1 drug resistance monitoring: nucleotide sequences of partial protease/reverse transcriptase and integrase genomic regions were determined by Sanger sequencing among treatment-naïve patients newly diagnosed with HIV-1 in Hungary between 2017 and 2024. Samples that yielded discordant results or were categorized as a rare circulating recombinant form (CRF) in the pol-based subtyping analysis were then subjected to NFLG sequencing with a newly developed protocol on Illumina Miseq platform. Recombination patterns of NFLG consensus sequences were determined using the recombinant identification program (RIP 3.0) and the jumping profile Hidden Markov Model (jpHMM) tool.

A total of 48 HIV-1 NFLG sequences were subtyped after genome assembly and consensus calling by the dshiver pipeline. Five sequences were identified as already characterized CRFs (CRF01_AE and CRF02_AG occurred in three and one sample, respectively), or pure subtype (subtype C in one case). Recombination analysis of the remaining 43 NFLGs revealed structurally diverse patterns of parental subtypes and recombination breakpoints, indicating the presence of possible unique recombinant forms (URFs) in Hungary. B/F1 intersubtype recombinants were observed most frequently (9/43, 20.9%) with different breakpoint positions, followed by recombinant HIV-1 strains with complex recombination patterns from at least 9 different parental subtypes.

This study provides new insight into the evolving genetic diversity of HIV-1 strains in Hungary. The novel URFs identified by NFLG sequencing emphasize the importance of ongoing molecular surveillance of the HIV-1 epidemic.

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Virology, -

In vitro promoter activity of human polyomavirus 9

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The in vivo cell tropism of human polyomavirus 9 (HPyV9) is still unknown. It is determined by the main surface antigen and the receptor usage, but after the entry, the steps of the replication within the nucleus are regulated by the noncoding control regions (NCCR) of the virus. The NCCR contains the origin of replication, and the bidirectional promoters and enhancers. The interaction between NCCR, cellular transcription factors, and viral regulatory proteins determines gene expression and whether latency or productive viral replication occurs in a cell. Therefore, an in vitro study of the promoter activity in different cell types may help to identify the possible permissive cells for HPyV9. The NCCR of HPyV9 has genetic variability, but its importance is still unknown.

The early and late promoter activity of HPyV9 and the effect of the large T antigen (LTAg) on it were studied in vitro in different cells using a bidirectional luciferase reporter vector. To study the biological importance of the rearrangement in HPyV9 NCCR we investigated the promoter activities of the reference genome and the rearranged UF-1 isolate. NCCRs were inserted into pGL4Luc-RLuc vector, a bidirectional reporter vector expressing firefly and Renilla luciferase in different directions under the control of NCCR. Normal, human primary airway epithelial cells, A549, HEK-293, MRC-5, Caco-2 and SK-HEP-1 cells were transfected. The promoter activities as luciferase activities were measured using the Dual-Luciferase Reporter.

We observed that both the early and late promoter activities of the reference and UF-1 NCCR were the strongest in the A549 and the weakest in the MRC-5 lung fibroblast line. Differences in early and late promoter activities were observed in different cell types. The promoters of UF-1 isolate showed stronger activities in almost all cells. LTAg significantly increased the late promoter activity. Our findings may help to determine the cell types permissive for HPyV9 replication. Although we observed low activity of the promoters in primary airway epithelial cells, the highest activity was detected in A549 lung cells, suggesting that the cells of the respiratory tract may be the site of HPyV9 replication.

Virology, -

Prevalence and genotype distribution of hepatitis B virus among pregnant women in Hungary

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Introduction: Hepatitis B virus screening of pregnant women began in Hungary in 1995 and continues to play an important role in preventing mother-to child transmission. The aim of our study was to assess the prevalence of HBsAg positivity and the distribution of HBV genotypes among pregnant women, with a particular focus on demographic changes in recent years.

Methods: Our laboratory received a total of 56,417 serum samples for prenatal screening in 2022 and 2023. We performed HBsAg ELISA as a screening test, and in positive samples viral DNA was detected using PCR. The resulting PCR products were sequenced and the viruses were genotyped. Additionally, sequencing and phylogenetic analysis were performed for 36 HBsAg-positive samples received for routine diagnostic testing.

Results: 56 of the pregnant women were confirmed to be HBsAg positive (0.1%). Based on names and/or OSZIR data, it was assumed that 25 of them were either not Hungarian citizens or were born outside Hungary. Thirteen of the HBV positive pregnant women were presumed to be from Southeast Asia, resulting in a prevalence of 4.3% (13/299) among this group.

Genotype determination and phylogenetic analysis were successfully performed for 36 of the HBsAg positive pregnant women and genotypes A2 and D1-3 were predominant. In nine cases, East Asian genotypes (B2, B4, C1) were found and these patients were of Chinese or Vietnamese descent. Among the samples received for routine testing, genotype D was the most common. Six samples contained Southeast Asian genotypes; however, in two cases, these did not originate from foreign patients.

Conclusion: The prevalence of HBV carriers among pregnant women in Hungary is low, but it is significantly higher among immigrants living in Hungary, especially those from Southeast Asia. This population carries genotypes B and C typical of Southeast Asia, while other groups almost exclusively carry the variants dominant in Europe. Our results indicate that HBV screening of pregnant women remains important. Targeted screening in immigrant and refugee populations may significantly contribute to reducing HBV prevalence and support the reaching of WHO's hepatitis B elimination goals.

Virology, -

Occurrence of recombinant canine coronavirus, picodicistrovirus and circovirus in red foxes (Vulpes vulpes) implies frequent virus transmission events among wild carnivores

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As several studies have described, some canine viruses could infect also wild carnivores. In this study coronavirus screening was carried out from saliva and fecal samples of 206 canid and mustelid animals in Hungary, such as red fox (Vulpes vulpes), European badger (Meles

meles), golden jackal (Canis aureus), stone marten (Martes foina), as well as raccoon dog (Nyctereutes procyonoides). Using broad-spectrum PCR, canine coronavirus (CCoV) sequences were identified in two red fox origin samples. Using next-generation metagenomics and direct sequencing, the near complete genome sequence was determined for these. Furthermore, near complete and whole genome sequences were obtained for a canine picodicistrovirus (CPDV) and a canine circovirus strain (CanineCV), respectively, in one of the samples. CanineCV has been found worldwide distributed, while CCoV has been identified in fox in China and Portugal. Regarding CPDV this the first noted appearance in Europe. The CCoV represented 94.1% and 94.3%, the CanineCV 96.2%, while the CPDV ≤87% genome-wide identity with the closest references. The results suggested that recombination could have an impact on the genomic evolution of wild carnivore origin CCoV, CanineCV and CPDV.

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Virology, -

Molecular epidemiology of respiratory syncytial virus among children in southern Hungary between 2017 and 2023

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Respiratory syncytial virus (RSV) is the most common lower respiratory tract pathogen in children under the age of 5 years and, therefore, represents a significant burden in health care every season. Our investigation aimed to analyse the prevalence of RSV infections between 2017 and 2023 in Hungary to determine the type of RSV in positive specimens, prepare a sequence variability analysis of the RSV attachment glycoprotein (G) gene, and investigate mutations in the epitopes of F protein.

Nasopharyngeal swabs from children under the age of 18 years with acute respiratory infection symptoms were tested primarily for RSV between 2017 and 2023. After determining the two main antigenic subgroups (RSVA and RSVB), we amplified the ectodomain of the G gene. We used overlapping regions based on literature data to determine sequence variations in the F protein gene. We tested 1831 samples for RSV during the examined periods. Two hundred thirty-three samples were positive for RSVA, 235 samples were positive for RSVB, and 5 samples were positive for both. In most seasons, type A dominated, except for the 2022/2023 season. The season 2021-2022 had an earlier onset of the RSV season and had a longer duration than before the SARS-CoV-2 era, similar to many European countries. Sequence analysis revealed that RSVA isolates belonged to ON1 subtypes between 2017 and 2023; these strains harbour duplications with minor modifications in the C-terminal domain of the G protein, as described earlier. Based on the nucleotide sequence of the hypervariable two regions of the G gene, domestic RSVB isolates can presumably be classified in the BA genotype. Similarly, RSVA show an insertion of 60 nucleotides, resulting in a longer G protein. Based on the sequence of the hypervariable section, the RSVB isolates from the local 2022 outbreak showed a close genetic relationship with 2022 isolates reported from several countries. We found characteristic mutations in the HRV1 region of the G gene in the RSVA strains during the 2019 and 2020 seasons, unlike international isolates except for two German

strains. The role of these mutations in virulence needs to be clarified. Compared to the G protein, the F protein is highly conserved, the sequence of its extracellular domain differs by only 5% in the case of the two subtypes (RSVA and RSVB), which explains the single RSV serotype. In our strains, the RSVB F protein epitopes had a higher polymorphism than the RSVA binding sites, also supported by studies conducted in the USA. The binding site of nirsevimab, a new single-dose monoclonal antibody approved by the Food and Drug Administration (FDA) in 2023, is highly conserved in domestic RSVA strains. However, we detected some amino acid substitutions in the RSVB isolates, the role of which is still unclear.

As mentioned above, RSV-associated respiratory infection is a significant morbidity in children under the age of 5 in Hungary. We confirmed RSV outbreaks over six years using sequence analysis of the G protein-coding gene. In the case of the ON1 and BA subtypes, the G protein-coding gene contains several sequence modifications in the C-terminal region of the protein in Hungarian isolates. Further studies are needed to clarify the role of variability in virulence affecting the G and F proteins, which may be enhanced by detailed knowledge of the clinical picture in the case of amino acid exchange carriers. Therefore, our studies include collecting clinical data for isolates.

Virology, -

DNA prevalence of human polyomaviruses in nasopharyngeal samples collected during COVID-19 pandemic

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The mode of transmission, the clinical importance, and the pathogenesis of human polyomavirus 9 (HPyV9), WU, KI, MW and STL polyomaviruses (WUPyV, KIPyV, MWPyV and STLPyV) are still unknown. Our aim was to investigate whether these viruses can be transmitted via respiratory secretions, and the possible co-infection with SARS-CoV-2. Nucleic acid extracted from nasopharyngeal swab samples sent for SARS-CoV-2 PCR to Medical Microbiology, University of Debrecen, Hungary, between 2020 and 2022, was analysed. The prevalence study was carried out with 919-1130 samples per virus. We compared the prevalence of polyomaviruses during and before the pandemic. Before the pandemic, our work team examined the prevalence of these viruses in respiratory samples from immunocompetent children and adults. Quantitative real-time PCR methods were used to detect polyomavirus DNA, and the positivity was confirmed using Sanger sequencing method.

All five viruses were detected in cohorts with a prevalence of 0.19–5.2%. No association between SARS-CoV-2 and any of the polyomaviruses was revealed. Polyomavirus DNA positivity rates were higher in SARS-CoV-2 RNA negative samples compared to SARS-CoV-2 RNA positive samples. WUPyV and KIPyV prevalence in samples from children was lower than expected. Although DNA prevalence among adults was not different from what was measured before the pandemic, we observed remarkably lower positivity compared to an Italian research team studying respiratory samples of people infected with SARS-CoV-2. We hypothesized that the time of sampling collection may explain the differences.

MWPyV and HPyV9 prevalence was higher than expected. MWPyV and STLPyV DNA positivity rates were significantly higher in samples from children than in samples from adults, while HPyV9 prevalence was higher among adults than children.

Based on our data, the respiratory transmission of the studied polyomaviruses are possible.

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Virology, -

Lymphocytic choriomeningitis virus infections in Hungary between 2017-2023 – investigation of the first congenital infections

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Background: Lymphocytic choriomeningitis virus (LCMV) is a neglected rodent-borne arenavirus, primarily spread by common house mouse species. The symptoms of infection are greatly influenced by the time of infection, so the clinical picture of pre- and postnatally acquired infection is completely different. Approximately one-third of acquired diseases are asymptomatic, or only mild, flu-like symptoms can be observed. Meningitis, encephalitis, or meningoencephalitis can occur in a more severe form of the infection. In contrast, intrauterine LCMV infection is associated with high mortality and morbidity. Infection of the fetus often leads to fetal death, and surviving fetuses may develop vision impairment (chorioretinitis) and central nervous system developmental disorders: microcephaly, macrocephaly, and hydrocephalus. Methods: Due to a short viremic period, LCMV is mainly diagnosed by serological methods using indirect immunofluorescence assays. LCMV nucleic acid detection by nested RT-PCR method was introduced in 2017, at the National Reference Laboratory for Viral Zoonoses. Results: In Hungary, 23 acquired lymphocytic choriomeningitis cases were diagnosed between 2017 and 2023. Ten out of 23 confirmed patients proved to be positive by the PCR method. Two intrauterine LCMV infections were detected in 2019 and 2021, respectively. The IgG antibody titers measured in the infant's serum samples were more than four-fold higher than the IgG titers of the maternal serum samples. In the first case, both IgM and IgA antibodies were detectable in the infants' sera. Conclusions: Despite the increasing number of cases reported in the literature, congenital LCMV infection might still be underdiagnosed. The microbiological diagnosis of LCMV is rather challenging because the symptoms are very similar to the clinical picture of other common teratogenic pathogens such as cytomegalovirus or Toxoplasma gondii.

Virology, -

New insights from the genomic study of Canine Distemper Virus

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The Canine Distemper Virus (CDV) is a single-stranded, negative-sense RNA virus, part of the Morbillivirus genus within the Paramyxoviridae family. This virus is highly lethal and affects both domestic and wild animals worldwide. CDV consists of multiple lineages, classified according to geographic location and based on nucleotide sequence analyses of the Hemagglutinin (H) gene. Although the hemagglutinin gene is frequently used to study CDV's genetic diversity, complete genome analysis offers a deeper understanding of the virus's evolutionary patterns. Complete genome sequencing can reveal specific mutations, recombination events, and evolutionary trends. CDV often jumps between species, posing significant conservation risks, especially for endangered animals. In Europe, CDV persists among various carnivores, where it leads to high mortality rates.

To investigate the long-term epidemiological trends of this highly pathogenic virus in wild carnivores, we conducted a different surveillance study. Samples from various species were analyzed, and viral genomes were sequenced using a pan-genotype CDV-specific amplicon-based next-generation sequencing method, utilizing Oxford Nanopore technology. Phylogenetic and recombination analyses were performed to understand the virus's genetic relationships. Our findings revealed the presence of CDV RNA in multiple small- to medium-sized carnivores across Europe. Phylogenetic analysis grouped the detected CDV sequences into the European and Arctic-like lineages, indicating the virus's continued circulation in European wildlife for the past two decades.

Complete genome data offer a more complete picture of the virus evolution than single-gene studies focusing on the Hemagglutinin gene. Additionally, long-term tissue sample collections present unique opportunities for retrospective pathogen analysis, contributing to conservation biology and ecological studies. Understanding infection events can support the conservation of endangered wildlife species and protect domestic animal populations from future CDV outbreaks.

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Virology, -

What is new for West Nile virus and Usutu virus in Hungary? – Overview of the 2024 seasonal period

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Introduction: West Nile virus (WNV) and Usutu virus (USUV) are closely related, endemic mosquito-borne orthoflaviviruses in Hungary. Altogether, 533 human WNV infections were laboratory-diagnosed between 2004 and 2023, whereas, only one human USUV neuro-

infection was confirmed in 2018. Most of the acute WNV-infected patients presented with moderate or severe neurological involvement, with up to 10% mortality rate of WNV-encephalitis. Materials and methods: The laboratory diagnosis of human infections is based on detecting virus-specific IgG, IgM, and IgA response in paired sera, using serological methods (e.g., IFA, ELISA). Differentiation from other endemic and/or imported orthoflavivirus-infections is essential, due to the serological cross-reactivity, the overlapping endemic areas, and the remarkably similar clinical symptoms. Whole blood and urine samples are the most suitable for molecular diagnosis and sequencing. Both Sanger-sequencing and NGS methods are used for virus typing. Virus isolation from acute-phase clinical specimens and PCR-positive mosquito pools was attempted on Vero E6 cell lines. Results: In 2024, the first autochthonous human WNV infection was recorded on July 24 (symptom onset: July 12), raising awareness for the early rise of WNV cases. Until August 12, 14 WNV neuro-infections were confirmed, exceeding the number of cases during the same period in 2023. One human USUV infection was also diagnosed using PCR method. The patient developed severe neurological symptoms and required intensive care treatment. Besides the laboratory detection of human infections, testing of Culex spp. mosquitos collected at seven sampling sites in Pest County, under the One Health SURVector project was also carried out at our laboratory. Until August 12, 273 mosquito pools were examined for the presence of WNV and USUV. Altogether, three mosquito pools were USUV-positive by real-time and nested RT-PCR, revealing active virus circulation. So far, USUV isolation on Vero E6 cell culture has been successful from one mosquito pool. Detection of new infections, virus isolation, and virus typing are still ongoing. The presentation aims to summarize the 2024 WNV/USUV seasonal period from a microbiological and epidemiological aspect.

Part of the study was performed in the frame of One Health SURVector project (Project number 101132974).

Virology, -

Tropical arbovirus infections imported into Hungary in 2024

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Introduction: Dengue virus (DENV), Zika virus (ZIKV) and Chikungunya virus (CHIKV) are among the most frequently imported tropical arbovirus infections in Hungary. Due to the overlapping endemic areas and similar clinical symptoms, differentiation of DENV, ZIKV and CHIKV should be based on microbiological methods. Materials and methods: The laboratory diagnosis of human infections was carried out by the detection of virus-specific IgG, IgM, and IgA antibodies from paired sera, using serological methods (e.g., IFA, ELISA), and viral RNA from serum, anticoagulated whole-blood and urine collected during the acute-phase of the disease. For laboratory diagnosis of DENV, NS1 antigen detection from serum collected up to 8 days after symptom onset was also performed. Virus isolation from acute-phase clinical specimens was attempted on Vero E6 cell lines. Results: Until 20th of August, altogether 179 patients were tested and 44 tropical arbovirus infections were diagnosed by laboratory methods. Most patients presented with fever, headache, exanthema and joint involvement. Similarly to the previous years, DENV was the most frequently imported tropical arbovirus infection. Most DENV cases were diagnosed between January and April, but unlike in the previous seasons, remarkably high number of DENV infections were detected during the

summer months as well. Moreover, most CHIKV cases were imported during summer. The majority of the tropical arbovirus infections were imported from Thailand, Indonesia and the Maldives. One co-infection of DENV and Parvovirus B19, and one co-infection of DENV and CHIKV were confirmed by PCR method. Detection of new infections, virus isolation, and virus typing are still ongoing.

Virology, -

MYPT1 is targeted by human papillomavirus E7

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Prophylactic vaccines have the potential to have a major impact on the global burden of human papillomavirus (HPV)-associated cancers, however, they have no therapeutic potential. Thus, for the development of therapeutic interventions, there is still a pressing need to understand the mechanisms by which these HPVs cause malignancy. A hallmark feature of HPV attributable cancers is the continued and high-level expression of viral E6 and E7 oncoproteins. These HPV proteins, particularly E7, play a significant role in induction of malignancy by targeting critical cell control pathways. Using mass spectometry analysis, we identified MYPT1, the targeting subunit of myosine phosphatase, as a novel interactor of HPV-16 E7 and PTPN14 protein complex. This creates the possibility that MYPT1 and, through this connection, MYPT1 regulated proteins can play a role in the life cycle of the virus and in the development of HPV-associated diseases. We aimed to characterize this association during our experiments.

First, we determined the expression levels of MYPT1 protein in several HPV positive and negative cell lines. Next, we investigated the effect of HPV E7 proteins from different genotypes (HPV-11, HPV-16, HPV-18) on the steady state expression level of MYPT1, and performed HPV E6/E7 specific siRNA treatment in HPV-18 positive HeLa and HPV-16 positive CaSki cells to investigate the effect of gene silencing on MYPT1 gene and protein expression. Moreover, we confirmed the interaction between the HPV E7 and MYPT1 by pull-down method.

We show that the presence of HPV E7 proteins leads to altered expression levels and cellular localization of MYPT1 protein, thereby possibly affecting its function. E7 oncoproteins of high-risk HPV types had prominent effect on MYPT1 protein expression, and HPV-16 E7 increased significantly MYPT1 gene expression. Moreover, we observed association between MYPT1 and HPV-16 E7 proteins, indicating MYPT1 being important for the function of high-risk E7 to enhance cell proliferation and induce malignancy.

Virology, -

Status of poliovirus containment and eradication, 2024

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In 1988, WHO initiated an intensive campaign to eradicate poliovirus, which has resulted in only two of the 125 endemic countries remaining endemic for wild poliovirus. Two of the three types of wild poliovirus have been declared eradicated (type 2: 2015, type 3: 2019).

Currently, vaccine-induced paralysis is the biggest problem, with 25 countries reporting cases last year. WHO recommends that OPV should be replaced by IPV in all countries, as complete eradication of poliomyelitis is possible only by eliminating the circulation of all polioviruses. WHO supports the introduction of new genetically stabilised poliovirus vaccines (e.g. nOPV2).

As part of the global poliovirus eradication programme, the WHO aims to drastically reduce the number of sites where potentially virus-bearing materials, wild virus and vaccine virus essential for vaccine production are stored. Hungary also strictly follows WHO guidelines in this area and was among the first countries to meet the requirements for the issuance of the ICC (Interim Certificate of Containment).

The WHO requires AFP surveillance: a sample from all children under 15 years of age with non-accidental paralysis must be sent to the National Poliovirus Reference Laboratory. The laboratory is responsible for the unequivocal identification and isolation of the poliovirus. Vaccination coverage and the availability of piped water and sanitation in Hungary meet WHO requirements.

Hungary is making every effort to achieve the polio eradication goals.

Virology, -

Effect of Avemar in feline AIDS model in vitro (Avemar hatása macska AIDS modellben in vitro)

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Avemar is an FDA-approved nutraceutical for the complementary therapy for several solid tumors and leukemias. This fermented wheat germ extract is produced by Saccharomyces cerevisiae, contains hundreds of molecules, among them the major biologically active ingredients are benzoquinone derivates. Both experimental and clinical studies prove that beneficial metabolic reversions, pro-apoptotic effects, diminishing metastases formation are coupled with restoration of immune functions. It alleviates side effects of chemo- or radiotherapy. So far, no studies have been conducted on the possible antiviral effect of Avemar. AIDS is a global health concern. Feline AIDS is the only natural small animal model to study pathomechanism and therapeutic interventions. We studied the in vitro effect of Avemar on feline immunodeficiency virus (FIV) and transactivating feline adenovirus (FeAdV). MBM lymphoid cells were acutely infected by European FIV-M2 and American FIV-Pet isolates. FL-4 cells continuously producing FIV-Pet served as a model for chronic infection. CRFK cells were infected by either FIV-Pet or FeAdV. Cultures were pre- and post-treated with serial dilutions of Avemar, subsequently residual virus infectivity was quantitated by FIV p24 and FeAdV hexon antigen, respectively, in time intervals. Low drug concentrations (<1000 microg/ml) temporarily slightly increased replication of feline lymphocytes (MBM, FL-4). In a concentration-dependent mode, Avemar inhibited replication of both FIV strains in MBM and CRFK cells by 3-5 log, finally cells lysed by day 17. Low drug concentrations (<500 microg/ml) prevented FIV release from FL-4 cells, but higher concentrations (>500 microg/ml) destroyed

virus-producing cells with cytopathic effects resembling apoptosis. Avemar pre-treatment significantly inhibited FeAdV production and virion release from CRFK but not in control cervical carcinoma HeLa cells. It is concluded that Avemar in a dose-dependent manner inhibits acute retrovirus replication and destroys infected cells. In chronic infections, phytate fragments of Avemar might interfere with budding process, but in a biphasic manner it elicits cell membrane rupture followed by release of entrapped FIV particles according to „lock-in and apoptosis" phenomenon. In HeLa cells, integrated HPV E6, E7 gene products transactivate FeAdV counter-acting with the effect of Avemar, but these cancerous cells finally undergo apoptosis. Extrapolation of these results suggest that in human and feline AIDS, Avemar or Immunovet, respectively, as non-toxic complementary therapy might exert anti-retroviral and beneficial effects on AIDS-related malignancies.

Virology, -

Bioinformatic analysis of open reading frames (ORFs) found in the long control region (LCR) of human alphapapillomaviruses

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Certain human papillomavirus (HPV) types belonging to the alphapapillomavirus genus are causative agents of cervical cancer. The expression of viral genes (including the E6 and E7 oncogenes) is governed by the long control region (LCR), which contains binding sites for viral (such as E2) and several cellular transcription factors. The 5' LCR region (lying between the L1 stop codon and the promoter-distal E2 binding site) contains the polyadenylation signal for the late viral transcripts, but it contains no known transcriptional enhancer sequences. Previously, we found that the 5' LCR of HPV 33 has an orientation-dependent toxic effect in certain E. coli strains used for cloning and this is probably caused by an open reading frame (ORF) potentially encoding a 116-amino acid protein. In this study, we explored if similar ORFs are found in other HPV types belonging to the alphapapillomavirus genus. Open reading frames (of a minimal length of 150 bp and with an ATG start codon close to the L1 stop codon) were found in altogether 42 of the 64 HPV types belonging to the alphapapillomavirus genus. The LCR sequences of the 42 HPV types were aligned and analysed by the MLOGD (Maximum-Likelihood Overlapping Gene Detector) software to get predictions about their coding capacity. The results of MLOGD analysis suggested that the ORFs found in the 5' LCR may have a coding potential. The putative proteins potentially encoded by these ORFs had similar amino acid composition with a high percentage of cysteine and hydrophobic amino acids (especially valine and leucine). Sequence alignment of the putative proteins revealed the presence of several conserved cysteine residues, but otherwise low sequence similarity. Protein structure prediction (performed by the I-TASSER and QUARK software) and amino-acid composition of these putative proteins suggest that they might be membrane proteins with a predominantly alpha-helical structure. We speculate that the conserved cysteines might stabilise the structure of these putative proteins allowing high sequence variation at other positions. Further studies should be performed to validate that these putative proteins are really expressed in naturally infected host cells.

Virology, -

Molecular epidemiology of the HIV-1 epidemic in Hungary until 2022

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We present the largest analysis of molecular epidemiological data on the HIV-1 epidemic in Hungary to date. Drug resistance genotyping started in smaller numbers in 2008 and was incorporated into routine clinical practice in 2016, yielding approximately 100 partial pol sequences from ~200 newly diagnosed cases per year over the last decade. In total we analyzed 1,645 partial pol sequences (889 from the PRRT and 756 from the INT region) from 892 patients up until 2022 to identify HIV-1 transmission patterns both within Hungary and across its borders.

First, we constructed maximum likelihood phylogenetic trees using the PRRT and INT sequence sets independently to identify long-term transmission clusters, employing several phylogenetic clustering approaches. Overall, we identified 25 sequence clusters containing at least five sequences each; 19 of these were predominantly subtype B. In all but one of these clusters, the MSM transmission group was the dominant component (>80%), indicating a significant separation between MSM and heterosexual risk groups. Members of larger clusters (10+ sequences) tended to be younger, more likely to be MSM, and had higher CD4 counts than patients not assigned to clusters.

Second, we applied a genetic distance-based network approach to detect rapidly expanding clusters indicating intense episodic transmission in recent years. We identified five large transmission clusters (with 20+ sequences), of which three MSM clusters showed substantial growth in 2021 and 2022, while the other two had become largely inactive by the end of 2020.

Finally, we extended our phylogenetic analysis by incorporating international HIV-1 sequences from public repositories to estimate the proportion of new infections that likely resulted from domestic transmissions versus newly introduced viral lineages. Overall, the number of clusters containing at least five sequences increased from 25 to 78. Among these, 16 clusters included only Hungarian sequences, four contained only international sequences, and 58 comprised a mix of both. Notably, three of the seven largest Hungarian clusters identified in the initial analysis also contained international sequences in basal positions on the phylogenetic tree, suggesting that these are recent introductions to Hungary that are now spreading within the country.

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Virology, -
CHAPTERS FROM THE EVOLUTION OF BAT CORONAVIRUSES

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Coronaviruses became one of the most important subjects for virologists dealing with zoonotic viruses after the SARS-CoV epidemic, nevertheless, they are in the focus because of the COVID-19 pandemic. With the sampling of Vietnamese horseshoe bats, 38 new Sarbecovirus genomes were identified, including relatives of SARS-CoV, SARS-CoV-2 and recombinants. One of these has the most similar Receptor Binding Domain (RBD) sequence to the SARS-CoV-2 known to date. The here determined sarbecoviruses composed two separate clades with the host species Rhinolophus affinis for SARS-CoV-2-like, and R. thomasi for SARS-1-CoV-like viruses. Our results show that the subtropical forests of South China, Northern Myanmar and Northern Vietnam are the cradle of SARS-coronaviruses.

In addition to Asian horseshoe bats, other hosts also carry coronaviruses that may be able to infect humans. A new coronavirus found in an Algerian bat has an RBD sequence and structure that should enable direct ACE2 binding with similar strength as the SARS- and SARS-CoV-2 RBDs. This also highlights the importance of studies discovering coronaviruses in new hosts in new areas.

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Virology, -
NOVEL ZOONOSES IN EUROPEAN BATS: RISK ASSESSMENT OF LLOVIU VIRUS AND RARE LYSSAVIRUSES

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Lloviu virus is a member of the Filoviridae family and the only known filovirus in European bats. Since its discovery, gathering evidence suggested the zoonotic nature of the virus and the possible pathogenicity to bats. Several important questions were raised during the last couple of years which we aimed to solve by sampling the currently known natural host of the virus, the Schreiber's bat. We collected more than 2000 blood samples and several hundreds of urine samples, feces, oral swab and ectoparasites. As a result of surveillance work, we have a picture about the shedding and annual patterns of the virus in these bats. In addition to

ecological examinations, we performed multiple pathogenicity studies in various animal models to better assess the pathogenic nature of the virus. In this presentation we summarize published and unpublished data about these experiments.

As an additional result we were able to identify seropositivity for two neglected Lyssaviruses, previously unknowns from our country. The West Caucasian bat lyssavirus (WCBLV) and Lleida bat lyssavirus (LLBLV) are considered as potential human pathogenic members of lyssaviruses. In an experiment, led by our partner institute, we were able to demonstrate the pathogenic nature of WCBLV but not LLBLV in a hamster model. Considering the results of the virological examination of Hungarian colonies of Schreiber's bat, we highlight the key elements of risk assessment to avoid possible human infections.

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Virology, -

Experimental inactivation of Zika virus using UV light

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Zika virus (ZIKV), a member of the Flaviviridae family, causes significant public health concerns. Although ZIKV typically causes flu-like symptoms, it can lead to serious neurological complications such as meningitis and Guillain-Barré syndrome. Pregnant women are especially vulnerable, as ZIKV can cause severe brain abnormalities in fetuses, including microcephaly. Currently, there is no available vaccine or specific treatment for ZIKV. Developing safe virus inactivation methods is essential for research and therapeutic purposes. This study examines the effectiveness of UV-C irradiation in inactivating ZIKV while preserving its immunogenic properties.

A custom-designed enclosed system was used to selectively expose ZIKV to UV-C light at a wavelength of 254 nm. The minimum irradiation time necessary to inhibit viral replication was determined via a TCID50 assay. Further characterization of the inactivated virus involved immunofluorescence assays targeting the and dsRNA to validate our inactivation and qRT-PCR to evaluate the integrity of the viral genome.

In our system, UV-C irradiation for 45 seconds successfully inactivated ZIKV, with no observable cytopathic effects, confirming the halt of viral replication. We looked at how different levels of irradiation affect the amount of active virus. Our results show a log-linear relationship between the irradiation level and the number of active viruses. The qRT-PCR results showed slight changes in the cyclic quantification (Cq) values. An irradiation time of 30 seconds shifted the Cq value by 1 cycle, indicating that the viral genome structure had changed slightly. Confocal microscopy revealed that although the inactivated virus can not replicate in the cell.

UV-C inactivation preserves the structural and biological integrity of ZIKV, making it suitable for applications in vaccine development, diagnostic assays, and antiviral research. This method offers a non-toxic alternative to chemical inactivation, making it safer for research involving pathogenic viruses.

UV-C irradiation has been established as a safe and effective method for ZIKV inactivation, enabling further exploration of virus-host interactions and vaccine development without the risk of infection. Future research will focus on expanding the applications of UV-inactivated ZIKV in therapeutic and diagnostic settings, as well as refining inactivation protocols to improve disease management strategies.

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