ABSTRACTS

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Comprehensive analysis of zearalenone exposure and its molecular effects in *Rhodococcus pyridinivorans* K404

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Zearalenone (ZEA) is an estrogenic mycotoxin produced by Fusarium spp., which poses both environmental and health risks due to its persistence and biological effects. In humans and animals, ZEA can disrupt hormonal balance and cause reproductive issues. This study examines the molecular responses and biodegradation capacity of the soil bacterium Rhodococcus pyridinivorans K404 upon exposure to ZEA, complemented by ecotoxicological assays to assess the residual toxicity of the degradation products. High-performance liquid chromatography (HPLC) analysis indicated a notable decrease in ZEA concentration after 48 hours, suggesting active transformation by the bacterium. At the same time point, acute toxicity testing using zebrafish (Danio rerio) demonstrated high remaining toxicity, with mortality reaching approximately 80%. These results suggest that a reduction in ZEA concentration does not automatically lead to full detoxification and emphasize the importance of combining molecular and ecotoxicological measurements in biodegradation studies. Transcriptomic analysis indicated a pronounced iron limitation and disruption of iron–sulfur (Fe–S) clusters. Genes involved in siderophore biosynthesis and uptake, iron-repair proteins, and manganese-dependent catalase were all upregulated, indicating a controlled oxidative stress response. Additionally, several enzymes with potential roles in direct ZEA detoxification were upregulated, especially an MBL-fold (Metallo-β-Lactamase fold) metallo-hydrolase, flavin containing monooxygenase, and F_{420}-reductase, Secondary metabolism-related polyketide synthases were also activated, suggesting that multiple metabolic pathways contribute to the bacterium's response to ZEA exposure. Based on transcriptomic data, six candidate genes (glyoxalase, amidohydrolase 3, MBL-hydrolase, flavin containing monooxygenase, Mn-catalase, and a siderophore-associated gene) were selected for targeted digital PCR validation. Together, these findings demonstrate that exposure to ZEA triggers complex responses in R. pyridinivorans K404, involving metal homeostasis, oxidative stress management, and xenobiotic detoxification pathways. Deciphering these molecular mechanisms, alongside ecotoxicological insights, paves the way for designing targeted strategies to neutralize the harmful impact of ZEA in food and feed.

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Investigation of spore surface proteins in *Mucor lusitanicus* to establish virulence factors

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Mucormycosis is a life-threatening fungal infection caused by Mucorales, predominantly affecting immunocompromised individuals and characterized by intrinsic resistance to most antifungals [1,2]. Spore coat proteins of the CotH family have been identified as key virulence factors: in Rhizopus delemar, CotH2 and CotH3 interact with host GRP78 to facilitate invasion [3,4]. Moreover, in Mucor

lusitanicus, at least two of 17 cotH-like genes have been implicated in normal spore morphology and virulence in murine and invertebrate models [5]. Here, we applied plasmid-free CRISPR-Cas9 technology to generate five targeted cotH knockout mutants in M. lusitanicus: MS12+pyrG ΔcotH13 to ΔcotH17. We conducted in silico analyses, in vitro assays, and in vivo studies using Drosophila melanogaster, Galleria mellonella, and J774.2 macrophage models to assess the impact of each deletion. The ΔcotH13 mutant exhibited significantly reduced virulence in both insect infection models with altered hyphal branching, indicating CotH13's role in structural integrity and pathogenicity. The ΔcotH14 strain displayed accelerated germination at 4 h and increased susceptibility to macrophage-mediated killing, suggesting CotH14 helps coordinate germination timing with immune evasion. Stress assays revealed differential phenotypes: ΔcotH13 showed enhanced resistance to Calcofluor White and SDS, ΔcotH17 was more sensitive to osmotic stress, while XTT assays demonstrated altered mitochondrial activity in ΔcotH15 and ΔcotH17. Additionally, ΔcotH16 spores displayed reduced survival post–macrophage interaction. These findings delineate specialized functions among CotH paralogs in spore viability, stress resistance, and virulence. Ongoing vertebrate animal studies aim to further validate the therapeutic targeting of CotH13 in mucormycosis control.

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Investigation of VSR coding capacity of apple infecting viruses

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Fruit trees are constantly exposed to viral infections. Persisting in the orchards for decades, they could become infected with several unrelated viruses. The host RNA interference (RNAi) based defence mechanism is activated during a viral infection. To evade this mechanism, viruses have evolved proteins that function as viral suppressors of RNAi (VSRs). Plant defence reactions can play a key role in the severity of symptoms. As they are affected by the efficiency of the VSRs, the severity of the symptoms can be directly connected to the presence and activity of VSRs. Recently, we have characterised the viromes of apple trees growing in Hungarian orchards using high-throughput sequencing (HTS) (Várallyay et al., 2022) and identified widespread infection with apple luteovirus 1 (ALV-1) and citrus concave gum viruses (CCGaV). ALV-1 (family Tombusviridae, genus Luteovirus), first identified in apple trees with rapid apple decline (RAD) disease, exhibits unique genomic features, including two ORFs (ORF0 and ORF5a) absent in other luteoviruses, however, familiar viruses encode VSRs at the 5' end of their genomes, in a region where ALV-1's ORF0 is located. The conservation of this putative P0 in all Hungarian ALV-1 variants suggests its potential role as a VSR. Additionally, the P4 protein encoded by luteoviruses, in the absence of P0, exhibits VSR activity. CCGaV (order Bunyavirales, family Phenuiviridae, genus Phlebovirus) was recently identified as the causal agent of citrus concave gum-blind pocket disease. The VSR activity of the coat protein (CP) of the Italian, citrus-infecting CCGaV variants exhibits a weak local VSR activity. This observation prompted an investigation into whether the Hungarian apple-infecting strains of CCGaV also possess VSR activity. In this study, we tested the RNA silencing suppressor potential of the P0 and P4 proteins of ALV-1 and the CP of CCGaV apple isolates. Our findings demonstrated that the P0 and P4 proteins of ALV-1 and the CP of CCGaV exhibited weak local and systemic VSR activity in transient assays. Investigations into their suppressor activity during mixed infections are currently ongoing.

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Evaluating the Potential of PGPR Strains to Enhance Growth and Drought Tolerance in Tomato and Maize Under Controlled and Field Conditions

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Tomato (Solanum lycopersicum L.) and sweet corn (Zea mays L.) are commercially and nutritionally important crops, whose productivity and resilience can be enhanced through environmentally sustainable agricultural practices. This study evaluates the efficacy of plant growth-promoting bacteria (PGPB) in enhancing crop performance and tolerance to water stress under both controlled and field conditions. Eleven non-pathogenic strains were selected from a bacterial strain collection originating from the rhizosphere of arid grasslands, based on key plant growth-promoting traits, including osmotic stress tolerance, IAA production, phosphate solubilization, siderophore and exopolysaccharide production and ACC deaminase activity. Controlled phytotron experiments were conducted to evaluate the short-term impact of these PGPB strains on the growth and physiological responses of tomato and sweet corn. Significant, crop-specific effects were observed. Notably, Pseudomonas, Kocuria, Brevibacillus and Stenotrophomonas strains significantly enhanced vegetative vigor in tomato, whereas a Brevibacillus strain was particularly effective in sweet corn. In contrast, a Pseudarthrobacter strain negatively affected sweet corn development. Based on these preliminary results, two strains - Kocuria sp. FSP120 and Brevibacillus sp. FSP5 -were selected for a greenhouse study to assess their longer-term effects on tomato under two irrigation regimes. Kocuria sp. FSP120 significantly improved several growth parameters, including plant height, number of leaves and flower buds. Under full irrigation, a 16% increase in marketable yield was recorded relative to the control, while an 11% increase was observed under deficit irrigation. Additionally, bacterial inoculation led to a measurable increase in fruit carotenoid content, indicating potential improvements in fruit quality. Currently, a field trial is underway to assess the performance of Pseudomonas sp. FSP16 and Stenotrophomonas sp. FSP12 on tomato development under 0% and 100% irrigation regimes

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Award-winning wines made with interspecific Saccharomyces yeasts

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In wineries, there is an increasing demand for dry yeast cultures suitable for special fermentation tasks, which can be used to safely produce premium, market-oriented alcoholic beverages. In addition, consumers prefer wines with fresh, fruity aromas, usually fermented at low temperatures.

At the same time, we are facing problems caused by climate change on several fronts: high alcohol content and higher pH, and the unfavorable aroma components in wines. We need to provide wineries with a starter culture that meets these needs. Although genetic modification technologies are at our disposal, the current regulatory background does not allow its application. A process that also occurs in nature among members of the Saccharomyces genus can solve the problem: interspecies hybridisation. We created interspecific hybrids in the laboratory: we crossed a lower alcohol-producing laboratory strain of S. cerevisiae (10-170) with the cryophilic wild S. uvarum (10-522), from a Tokaj winery. Their hybrids' real hybrid nature was confirmed by karyotyping: both parental chromosome sets were detected by CHEF. We also examined the RFLP profile of the mitochondrial genome of our hybrids. Based on the results, we selected recombinant type and parental mitotyped hybrids because we were also curious if the mitochondrial genome had any effect on the fermentation ability of the hybrids. We performed an experimental fermentation in pasteurized Kékfrankos grape must. We used a commercial starter culture (Mycoferm CRU-31) as a control and examined the selected strains and their parental strains. The dynamics of the fermentations were monitored by measuring the decrease of sugar content. The classical analytical parameters of the finished wines were determined by FTIR spectrometry, and the volatile compounds were determined by headspace gas chromatography-mass spectrometry. The sensory evaluation of the wines was carried out by a five-person expert group. The low alcohol production trait of the S. cerevisiae strain was fully complemented by the S. uvarum strain. All hybrid strains produced more alcohol than the parental strains. Two of them achieved higher alcohol levels than the commercial strain. Two wines, fermented by hybrids scored higher in sensory evaluation than the control wine fermented with the commercial starter culture. Using gas chromatography, we were able to separate more aroma components in the wines fermented with the hybrid strains than in those fermented with the parental strains. With our finished Kékfrankos rosé wine, we participated in the 13th International University Wine Competition in Meranovo, Slovenia, where our experimental wine won third place in the rosé category.

Enhancing Strain Discrimination in *Klebsiella pneumoniae* MALDI TOF MS Typing through Batch Effect Correction

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Matrix-assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI TOF MS) has become a critical tool in clinical diagnostics and research due to its speed and low cost. Batch effects that arise from technical and biological variations during analysis pose a challenge in microbial datasets, possibly leading to false clustering. This study focused on correcting batch effects that arose from culture conditions, matrix composition and time of measurements. Raw spectra from MALDI measurements were preprocessed in R and batch effect removal was performed using ComBat in the R program. Analysis of preprocessed spectra utilized both Principal Component Analysis (PCA) and sparse Partial Least Squares Discriminant Analysis (sPLS-DA) to identify discriminant features in the 24 Klebsiella pneumoniae isolates included in this study. The isolates belonged to sequence types (STs) 15, 101 and 147. In order to support batch effect removal, protein extraction pooled samples for quality control (QC) were included and the position of the samples on the MALDI target plate was randomized. The PCA before batch effect removal showed that clustering was based on time of measurement and not Klebsiella pneumoniae strains. After batch correction, PCA plots revealed a more uniform distribution, indicating a reduction in variation and a better representation of biological variability. To get the discriminatory features for further classification of the isolates, a tuned sPLS-DA was employed. The resulting sPLS-DA score plots separated the isolates into three

groups that corresponded to the STs (15, 101 and 147) with only two mass spectrum from ST 15 being misassigned. Heat map highlighted influential features (2678 – 11900 m/z) that contributed to this discrimination. Combining the use of QC samples and randomization offers an effective strategy for batch removal, thereby enhancing data quality and enabling the identification of biologically relevant features for strain differentiation. These findings underscore the value of QC samples and combat R for batch removal, offering an enhanced methodology for diagnostics and biomedical research.

Unraveling native microbial communities and bioremediation dynamics in hydrocarbon-contaminated aquifers: evidence for site-specific adaptation

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Understanding how native microbial communities respond to bioremediation interventions is critical for optimizing the clean-up of hydrocarbon-contaminated aquifers. This study presents four years of high-resolution microbial monitoring across multiple wells in a field site historically impacted by BTEX compounds. Using 16S rRNA gene sequencing, we tracked shifts in community structure and diversity to evaluate bioremediation progress. Sequencing revealed that while a core set of phyla—primarily Pseudomonadota, Bacteroidota, and Bacillota—persisted throughout the study, their relative abundances changed markedly over time. Early stages were dominated by classical hydrocarbon degraders such as Pseudomonas, Rhodoferax, and Zoogloea, reflecting high contaminant loads. As remediation progressed, these genera declined, while anaerobic fermenters and syntrophic taxa, including Lenti-02, Synergistaceae, and Rectinema, emerged, indicating functional transitions and reduced contamination stress. Site-specific trends highlighted distinct microenvironments within the aquifer. PI2 and PI6 showed clear signs of recovery, with increased community evenness and emergence of diverse fermenters, pointing to stabilization and functional redundancy. In contrast, PI7 exhibited persistent community instability, suggesting unresolved redox fluctuations. Overall, our findings demonstrate that native microbial communities are robust drivers of in situ bioremediation, and that long-term molecular monitoring is invaluable for capturing these ecological successions. These insights emphasize the need to harness native microbiomes for sustainable remediation and highlight the potential of future functional and cultivation-based studies to expand bioremediation capabilities.

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Comparative evaluation of BioFire® FilmArray® Panels and conventional culture for rapid microbiological diagnosis

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Rapid and accurate identification of microbial pathogens is critical for effective clinical decision-making, particularly in life-threatening infections such as sepsis, meningitis, and lower respiratory tract infections. Traditional culture-based diagnostic techniques, long considered the gold standard

in clinical microbiology, are inherently time-consuming, often requiring 24–72 hours. In contrast, recent advances in molecular diagnostics, especially multiplex PCR-based systems such as BioFire and QIAstat-Dx, enable direct detection of pathogen-specific nucleic acids from clinical specimens in about one hour. This study compares the performance and limitations of these approaches. Multiplex PCR systems allow rapid identification of predefined bacterial, viral, and fungal pathogens directly from blood, cerebrospinal fluid, or respiratory secretions. Their high analytical sensitivity enables detection of low-abundance, fastidious, or slow-growing organisms and certain antimicrobial resistance genes (e.g., mecA, CTX-M, NDM), providing early therapeutic guidance. However, fixed panels cannot detect unexpected or rare organisms outside their target list, and residual DNA from treated infections may yield false positives. Between 2022 and June 2025, 1,391 BioFire multiplex PCR panels were performed, rising from 174 tests in 2022 to a peak of 567 in 2024. The most common panels were lower respiratory (n=395), meningitis (n=392), and upper respiratory (n=229), followed by gastrointestinal (n=198), joint infection (n=111), and blood culture identification (n=66). Relative to total laboratory requests (56,768 in 2022; 70,189 in 2024), multiplex PCR usage increased from 0.31% to 0.81%, a statistically significant rise ($p < 0.0001$). Conventional culture methods offer broad, untargeted recovery of viable pathogens, including polymicrobial infections, and enable phenotypic susceptibility testing and further characterization. They also help distinguish pathogens from commensals based on colony morphology, but their slower turnaround can delay therapy. In summary, rapid molecular diagnostics have increased markedly in recent years, reflecting broader clinical adoption. Nevertheless, culture-based diagnostics remain indispensable for comprehensive microbial assessment and antimicrobial susceptibility testing, and the integration of both methods ensures the most reliable and timely patient care.

New biological solutions by the application of bioactive peptaibols

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The agricultural and biotechnology industry need innovative and effective biological solutions against extreme weather conditions and spreading new pathogens. Small bioactive peptides, like peptaibols, produced by filamentous fungal Trichoderma species can provide new opportunities due to their stability and characteristic properties. In addition to their antagonistic effect against plant pathogenic microorganisms, they may have a positive effect on plant development, which can establish their use as a multifunctional product. However, for their practical application, their comprehensive investigation is inevitable, and the development of new methods are needed for their large-scale and sustainable production. In our work, we perform the comprehensive investigation of the total peptaibol production of Trichoderma species (peptaibiome) by analytical methods. Furthermore, through laboratory studies, we gain information about the effect of peptaibols against microorganisms, while with the application of computational molecular modelling techniques, we can gain a deeper understanding of the background mechanisms of their action and structure. Based on our results, we can select the peptaibol mixtures with the most suitable properties for large-scale production, and investigate the effects of the extracts on agriculturally significant plants. Our main goal is to identify new peptaibols and establish their effective practical application.

The Tree Phyllosphere Microbiome: An Overlooked and Important Sink for Carbon Monoxide?

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Carbon monoxide (CO) is a colourless, toxic gas that influences atmospheric chemistry by extending the lifetime of major greenhouse gases such as methane. Microbial utilisation of CO as a carbon and/or energy source contributes significantly to global carbon cycling. Recent studies indicate that tree phyllosphere microbiomes harbour diverse CO-degrading bacteria; however, the physiological role of CO degradation and the enzymes and genes underpinning CO oxidation in these bacteria remain unclear. To address these gaps, we employed both culture-based and culture-independent approaches. Holly leaf microbiome samples were enriched under different conditions, with CO concentrations of 100 ppmv and 10,0000 ppmv, to examine the phylogenetic and functional diversity of CO-oxidizing bacteria. Several CO-degrading strains were isolated from the enrichments and their genomes sequenced, including a strain belonging to a putative novel genus containing only form II CODH, which was capable of CO oxidation. Additionally, we identified a *Pseudomonas* strain SB113 that oxidised CO across a wide concentration range (100 ppmv – 10,000 ppmv) despite lacking any known carbon monoxide dehydrogenase (CODH)-encoding genes. Metagenomic analysis revealed that CO concentration significantly influenced microbial community composition and *coxL* gene diversity. CO oxidation by strain SB113 in the absence of a detectable *coxL* gene suggests the presence of a hitherto unidentified enzyme degrading CO, a hypothesis further supported by proteomic data. This study provides novel insights into the metabolic diversity and genetic basis of CO oxidation in phyllosphere bacteria. These findings suggest that our current understanding of microbial CO cycling is incomplete, highlighting the need for further exploration of alternative CO oxidation pathways.

Climatic predictors as early warning signals for West Nile virus outbreaks

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West Nile virus (WNV) has been present in Croatia for more than a decade, with human cases reported almost every year since 2012. To capture the temporal dynamics, this study focused on the period from 2015 to 2019, which was deliberately selected to include 2018, the year with the highest number of reported human cases. The dataset included IgG seroprevalence in horses, acute infections in horses (IgM-confirmed), reported human cases, and key climatic variables in eastern and central Croatia. Meteorological data (precipitation, relative humidity, and temperature) were obtained on a monthly and annual basis by the Croatian Meteorological and Hydrological Service at four stations, two of which were located in eastern and two in central Croatia - regions chosen based on the continuous confirmation of human cases. Statistical analyses revealed that inter-annual variations in abiotic factors had no consistent influence on virus activity, while temperature trends analysed at the monthly level were significantly associated with the occurrence of human cases and acute infections in horses. Above-average temperatures in April increased the probability of human cases in the same year by 1.96-fold ($p = 0.02$), while above-average temperatures in May increased the probability by 2.48-fold ($p = 0.01$). A similar trend ($p = 0.05$) was observed for acute equine infections, with the probability of infection increasing 1.67-fold in April and 1.69-fold in May when temperatures were above average. These results emphasise the importance of complementing

surveillance of West Nile infections in sentinel animals by analysing climate data to improve the public health early warning system and increase preparedness for future WNV outbreaks.

Role of the Heat Shock Transcription Factor HSF-1 in the Intracellular Pathogen Response of the Nematode *Caenorhabditis elegans*

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HSF-1 is an evolutionarily conserved transcription factor that plays a central role in the heat shock response. Beyond this canonical function, HSF-1 is essential for key biological processes including development, metabolism, and aging. It is also implicated in the pathogenesis of several diseases, notably neurodegenerative disorders and cancer. Our RNA sequencing results revealed a paradoxical finding: both activation and inactivation of HSF-1 in Caenorhabditis elegans lead to the induction of genes involved in innate immunity. While HSF-1 has previously been shown to promote innate immune responses in nematodes, immune activation in its absence had not been described before.

To investigate this phenomenon, we employed comparative transcriptomic analysis using published RNA-seq datasets, focusing on the immune response triggered by decreased HSF-1 activity. We further validated the upregulation of specific immune genes through quantitative real-time PCR and GFP reporter assays. Our findings demonstrate that the intracellular pathogen response (IPR), typically activated by viral infections and intracellular parasites, is induced in the absence of HSF-1. Importantly, our data suggest that HSF-1 does not directly repress this pathway. Rather, the activation of the IPR appears to be a secondary consequence of proteotoxic stress resulting from reduced HSF-1 activity. We are currently investigating whether modulating HSF-1 activity affects C. elegans tolerance to intracellular pathogens such as the Orsay virus and the microsporidian parasite Nematocida parisii.

Screening and Characterization of Probiotic Microorganisms in the Nematode Caenorhabditis elegans

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The gut flora refers to the diverse community of microorganisms residing in the intestinal tract. These microbes play essential roles in numerous biological processes and influence others through complex, still not fully understood pathways. Symbiotic bacteria are especially important for the synthesis of certain vitamins and for supporting various immune functions. The microbiome has also been implicated in the development of some cancers and neurodegenerative diseases. Moreover, changes in the composition of gut flora during aging may influence lifespan. Caenorhabditis elegans is a widely used model organism for screening probiotic bacteria. Its low maintenance cost, transparent body, and short lifespan make it a more practical laboratory model than mice, which are more expensive and more challenging to keep germ-free. The aim of this research is to screen and characterize probiotic bacterial strains from a well-defined collection of several hundred Lactobacillus isolates of diverse origin, established by József Kukolya and colleagues. Using the Caenorhabditis elegans model, we seek to identify microbial strains that can slow aging, enhance stress resistance, and ameliorate symptoms of neurodegenerative diseases. To achieve this, we are

developing an optimized protocol for the rapid and efficient identification of bacterial strains with probiotic activity. Among the strains tested so far, one isolate—designated AT51 and originally isolated from the intestine of the Aldabra giant tortoise (Aldabrachelys gigantea)—has significantly extended the lifespan of C. elegans compared to worms fed the standard laboratory diet of E. coli OP50. We are currently investigating the mechanisms underlying the lifespan-extending effects of strain AT51 by analyzing the expression of various reporter genes and examining the phenotypes of mutants impaired in key pathways regulating longevity and stress response. Furthermore, we are evaluating the potential neuroprotective effects of this strain in C. elegans models of neurodegenerative diseases.

Exploring LAB as natural inhibitors of *Clostridium difficile*

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Clostridium difficile is a significant pathogen associated with healthcare-related infections and gastrointestinal disorders, particularly in hospitalised and immunocompromised patients. Despite its clinical importance, effective and universally applicable treatments remain limited. In this study, we investigated the antagonistic activity of 108 lactic acid bacterium (LAB) strains from our in-house collection, encompassing six different Lactobacillus species, against C. difficile. Co-culture assays were employed to assess inhibitory interactions between the LAB strains and a reference C. difficile strain. Notably, only 14 LAB strains showed no inhibitory activity, including all representatives of Lactobacillus amylotrophicus and Lactobacillus amylovorus. The most pronounced antagonistic effects were observed in strains belonging to Lactobacillus mucosae, Lactobacillus johnsonii, Lactobacillus kitasatonis, and Lactobacillus brevis. Interestingly, intra-species variability was evident, with up to 30% variation in inhibition efficacy among strains of the same species. These findings highlight the potential of specific LAB strains as candidates for probiotic-based interventions targeting C. difficile, while also emphasising the need for strain-level evaluation rather than relying solely on species identity. This work contributes to the development of targeted microbiome therapies and underlines the importance of precision selection in probiotic research.

Correlating mortality peaks with respiratory virus emergence: a blueprint for identifying historical pandemics

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Just as SARS-CoV-2 is transforming into a seasonal respiratory pathogen, the causative agents of other seasonal respiratory infections may also have caused pandemics with substantial global mortality when they first entered the human population. We aimed to identify the footprint of such historical pandemics in historical mortality data. We extracted historical mortality data from the Human Mortality Database. For broadest possible coverage and due to the uncertainty of causes of death in the historical data, we chose to analyze all-cause mortality in the datasets. We defined peak mortality years as years that had simultaneous peaks of mortality in several countries using various coincidence metrics. We compiled published estimates on the most recent common ancestors (tMRCA) of common respiratory viruses and used expert opinion to select likely best estimates.

Finally, we defined potential pandemic years as peak mortality years that fell within the tMRCA range of at least one respiratory virus. We identified 11 common respiratory viruses that had tMRCA estimates overlapping with the range of historical mortality data (3 or more country datasets from 1835). We selected coincidence metrics that reliably highlighted known historical influenzavirus pandemics (1918, 1957, 1968) as local peaks of combined (multi-country) mortality. We present a list of potential pandemic years that coincide with the estimated origin of one or more seasonal respiratory viruses. While the uncertainty in the tMRCA estimates precludes the unique association of each peak mortality year with a single pathogen, potential pandemic years could be identified for the majority of current seasonal respiratory viruses. We argue that although all-cause mortality has multiple components, few causes other than global epidemics can generate mortality peaks spanning many countries. The potential pandemic years identified in our analysis can motivate targeted validation efforts and contribute to a better understanding of the emergence of new pandemics.

Bacteriophages and black rot of brassicas: Towards alternative control strategies

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Black rot of brassicas, caused by Xanthomonas campestris pv. campestris (Xcc), is one of the most destructive diseases of cruciferous crops worldwide. The pathogen infects a wide range of economically important crops, including cabbage, cauliflower, and broccoli, causing significant yield losses. In recent years, black rot has also become a significant and growing problem for commercial cabbage producers in Slovenia. With the increasing severity of the disease and the lack of effective control agents, bacteriophages are gaining recognition as a promising alternative in the management of bacterial plant diseases. With this aim, we isolated bacteriophages infecting Xcc from various environmental samples from Slovenia. We implemented methods to assess the physiological characteristics and stability of the phages, as well as DNA isolation method for their genetic characterization. The obtained characteristics allow meaningful comparison between phages and provide essential information for their potential application in the control of Xcc. Host range, one of the most important characteristics for the application of bacteriophages, also provided insight into differences among the phages and, for the first time, between Xcc isolates that we isolated from various infected brassicas in recent years.
This work was founded by the Ministry of Agriculture, Forestry and Food of Slovenia, and Slovenian Research and Innovation Agency (ARIS Program P4-0072 and P4-0431).

Detailed phenotypic characterization of lytic enterobacterial phages isolated from Hungarian livestock

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Bacteriophages are increasingly studied as potential antibacterial agents effective against enterobacterial pathogens. With E. coli K-12 strain MG1655, enterohemorrhagic E. coli O157:H7 Sakai strain, and Citrobacter rodentium strain ICC169, we isolated 140 lytic phages from animal faeces collected from Hungarian livestock. Host spectrum experiments were conducted using E. coli strains representing various pathotypes. Seven phages were chosen for further detailed phenotypic

characterization. Phagescore quantitatively shows the lytic activity of the phage against a given host bacterium. Phagescore values of the studied phages on their original hosts used for isolation ranged from 0.0025 to 0.0657. When testing their thermal tolerance, compared to the 4°C control, phages did not survive incubation of 1 hour at 80°C, except for one phage that retained 50% of its activity. At 1 hour 60°C treatment, the activity of three phages was almost zero, while three phages retained 20-40% of their activity, one phage remained almost the same (-4%). After 24 hours at room temperature four phages retained 60-80% of their acivity, two phages performed. +8-10%, one phage reached 4x acivity. When simulating abuse temperatures by incubating phage samples for two hours at room temperature, then cooling back to 4°C, the majority of phages showed increased activity; one phage increased drastically; 5x activity (it was the same phage wich increased 4x activity in 24 h RT), another reached +115%, three phages showed a 4.5-9.5% improvement, and the remaining two phages experienced a 30-40% decrease. In the pH tolerance experiment, compared to neutral pH, no phage survived pH 1, while at pH 5, survival of 3.5-5% was observed in two cases, 30-35% in another two cases, survival above 60% in two cases and a 50% titre increase in one case. At pH 9, five phages showed no substantial decrease (-33%, -18%, three of them less than -10%), one of them showed a 36% improvement, and a 150% improvement in one phage was observed. At pH 13, all phages showed increased activity at least 28%, up to 158%. Overall, we found no correlation between phagescore, heat and pH tolerance, the pattern was unique for each phage, however, the tested phages tolerated alkaline conditions and heat better than expected, which may be beneficial in terms of their combined applicability with other active ingredients/treatments.

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Detection of ica and bap genes related with biofilm-forming ability in staphylococci from ewe milk lump cheeses

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The genes ica and Bap are associated with biofilm-forming ability in staphylococci. Biofilm formation belongs among one of virulence factors. It means that staphylococcal strains containing these genes can cause as disorder or damage stimulating agents. Therefore, it is necessary to check staphylococci for virulence factor genes to know their pathogenic potential. Because sheep breeding has a long tradition in Slovakia, ewes milk and products made from it by the traditional way are popular among consumers. That it is needed to know potential contaminats such as staphylococci are and also to look for their prevention and/or elimination when they are occurred in tested niches. In this study were tested various staphylococcasl species isolated from ewes milk lump cheeses for the presence of those ica and Bap genes. Thirteen different staphylococcal species strains were analyzed such as Staphylococcus xylosus, S. succinus, S. simulans, S. equorum, and S. aureus. Seven (7) strains out of 13 were genes absent and they were from the species S. xylosus, S. aureus, S. succinus, and S. simulans. Five (5) out of those 7 both genes absent strains were found with low-grade biofilm-forming ability (0.103-0.170). Ica gene was present in 4 out of 13 strains. Bap gene was found in 3 species strains. Both genes containing strain S. aureus SAOS6 did not form biofilm. It looks that analyzed staphylococci did not represent real threaten. In addition, use of postbiotics (bacteriocins-enterocins) has been shown as effective treatment to inhibit them.

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Association between virulence factors and transmissible pathogenicity potential in enterococci from wild roe deer

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Microbial communities inhabiting the gastrointestinal tract are essential for host health, contributing to metabolic functions, immune system modulation, and resistance to pathogens. Despite growing interest in the intestinal microbiota of domestic animals, knowledge about microbiota associated with wild animals remains insufficient. This study investigates faecal enterococci from free-living (wild) roe deer (Capreolus capreolus) in eastern Slovakia with the aim to expand our knowledge regarding the microbial ecology of wild game species and their potential role as reservoirs of pathogenicity based on the biofilm-forming ability genes detection and other virulence factors. Mixtures faecal samples (20) were collected from wild roe deer in the Zádiel area of eastern Slovakia and then transported to the laboratory for further analysis. They were processed using a standard microbiological method according to ISO and inoculated on various selective media. For enterococci was used M-Enterococcus agar (Difco, USA). Enterococci were identified using mass laser spectrometry (MALDI-Tof MS). Subsequently, the presence of virulence factor genes (gel, esp, cyl, efa, agg, hyl, IS16, ccf, cpd) was analyzed using PCR. Among isolated faecal strains, 25 enterococci were identified. Based on the species identification, the strains were allotted into three species: Enterococcus faecalis (9 strains), Ent. mundtii (15 strains) and Ent. casseliflavus (1 strain). The most frequently detected gene was esp. It was present in 16 of 25 strains (64%), predominantly in strains of the species Ent. mundtii (14 of 15). The ccf and hyl genes were recorded in 13 strains (52%); the gel and cpd genes were found in 11 strains (44%), and the cyl gene, associated with significant pathogenicity, was not found in any of the analyzed strains. Test results showed that enterococci isolated from the faeces of wild roe deer carry several genes associated with virulence, particularly the esp, gel, hyl, and IS16 genes. Ent. faecalis strains exhibited complex virulence profiles similar to those characteristic of clinically significant strains. The results point to the need for further research and monitoring of the wild animals microbiota in the context of the One Health concept.
The study was financially supported by the projects Vega 2/0009/25 and COST22166.

The effect of short and long-term water deficit on physiological performance and leaf microbiome of different rootstock and scion combinations

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Climate change, particularly drought stress, threatens viticulture sustainability. Understanding scion-rootstock interactions and their link to the grapevine microbiome is key to improving vine health, productivity, and drought resilience. The plant mycobiome, through its interactions with the host plays important roles in plant health and in the quality and quantity of crops. Our work had main objectives to study the effect of short and long-term water deficit on the leaf microbiome of Furmint varieties grafted on different rootstocks. This experiment, we conducted a pot experiment with Furmint grafted onto Teleki 5C and Richter 110 and Paulsen1103 rootstocks, which differ in drought tolerance. Plants were maintained either at 50% field capacity (water deficit) or 100% field capacity (well-watered control). Drought stress was imposed for 14 days (short-term) and 42 days (long-term), followed by re-watering in both cases. In parallel, we sampled leaves from additional rootstocks for DNA extraction (Teleki 5C, Richter 110, and Paulsen 1103) and profiled leaf-associated fungal

communities using ITS2 rDNA metabarcoding. Reads were quality-filtered and denoised with dada2 to infer sequence variants, taxonomically identified via pairwise similarity searches against the UNITE database using USEARCH, and functionally annotated with FungiTraits. To minimize terroir- or cultivar-specific signals, we retained only taxa detected across all sampled terroirs and prevalent in most cultivars. This design enables robust testing of rootstock- and water-status effects on the grapevine leaf mycobiome. Leaf-inhabiting fungal communities displayed distinct patterns across rootstocks. The richness and relative abundance of fungal functional groups differed significantly in both drought-stressed and rewatered samples. The community composition of the dominant functional groups—i.e., leaf-associated plant pathogenic fungi—also differed significantly among treatments. Our results indicate that drought stress and water deficit strongly shape the grapevine leaf mycobiome; elucidating the causal mechanisms will require targeted studies that could meaningfully contribute to mitigating challenges posed by global warming.

Detection of SDHI fungicide resistance markers in *Podosphaera xanthii* infecting cucurbitaceous crops in Hungary

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Powdery mildew is one of the most frequently occurring diseases of cucurbit crops. Succinate dehydrogenase inhibitors (SDHI) are widely used to control diseases, but their effectiveness is decreasing due to the increasing spread of fungicide resistance. Our aim was to identify the powdery mildew species that infect cucurbit crops and to assess the presence of markers associated with resistance to SDHI fungicides. We sampled over thirty plots, collecting samples from various species and cultivars of cucurbits, watermelon, and cucumber. To identify the fungi, we sequenced the internal transcribed spacer region of the ribosomal DNA and developed a restriction digestion-based method for the rapid differentiation of the two identified species. Fungi on approximately two-thirds of our samples belonged to the Golovinomyces orontii s. lat. species complex, while in other samples, we identified Podosphaera xanthii. From the P. xanthii samples, we sequenced the sdhB, sdhC and sdhD genes, which encode three subunits of succinate dehydrogenase and are known to carry markers of resistance to SDHI fungicides. All determined sdhD sequences were identical to wild-type reference sequences. However, in the coding region of the sdhC gene, we identified a G-to-A nucleotide substitution at position 612 (G612A), which results in an amino acid substitution, denoted G151R. In the coding region of the sdhB gene, we identified a A-to-G nucleotide substitution at position 901 (A901G), which results in an amino acid substitution, denoted H243R. G151R has been detected in P. xanthii samples from Japan and Spain and is associated with resistance to several SDHIs. H243R has been detected in P. xanthii samples from Japan and homologous SNPs have been reported from various SDHI-resistant pathogens. Our study is the first to report these mutations in Hungary. Our data highlight the importance of local monitoring of fungicide resistance markers and have the potential to support disease management in cucurbit production.

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Exploring the Oenological Potential of Non-Conventional Yeast Strains in Apple Juice Fermentation

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Yeasts have been used in food and beverage production for millennia, particularly in breadmaking and alcoholic fermentation. While Saccharomyces cerevisiae and Saccharomyces bayanus are well-established in wine and beer production, there is growing interest in utilizing non-conventional yeast strains—particularly in the craft beverage sector—due to their potential to impart unique sensory characteristics. Our study focused on evaluating various strains from the Schizosaccharomyces genus (S. pombe, S. pombe var. malidevorans, S. japonicus, S. octosporus, S. cryophilus) concerning their oenological properties, including sugar assimilation, fermentation performance, alcohol tolerance, and sulfur tolerance. One of our primary objectives was to identify optimal culture conditions for each strain by determining their sugar requirements, temperature, and pH preferences. In initial experiments, apple juice from Malus domestica 'Golden Delicious' was fermented. Small-scale laboratory fermentations were conducted, and fermentation kinetics were monitored. Each strain exhibited distinct fermentation dynamics and efficiency. Upon completion, the fermented juices were analyzed for alcohol content, residual sugars, and organic acids (acetic, citric, malic, tartaric, and lactic acid). Aroma compounds were profiled using gas chromatography. While most strains produced aroma profiles similar to the control, each also contributed unique volatile compounds to the final product. Subsequent fermentations were carried out using ‘Jonathan’ and ‘Jonagold’ apple juices and selected yeast strains (S. pombe var. malidevorans, S. japonicus, and S. bayanus as controls). We observed that not only the yeast strain but also the apple variety influenced fermentation kinetics and the chemical composition of the final product. Notably, S. pombe var. malidevorans and S. bayanus produced more similar aroma profiles compared to S. japonicus, although all three were characterized by fruity and floral aromatic compounds. In conclusion, our results demonstrate that non-conventional yeast strains—especially those from the Schizosaccharomyces genus—have promising potential in fruit juice fermentation. These strains can compete with traditional yeasts in terms of fermentation efficiency, analytical parameters, and aromatic complexity, offering new possibilities for innovation in craft cider and alcoholic beverage production.

Investigation of the microbiological efficiency of poultry manure-based compost tea; metagenome analysis, and identification of microorganisms that can be cultivated

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The European Union's “Green Deal” agreement aims to reduce the use and risks of chemical pesticides. The European Commission plans to have at least 25% of EU farmland under organic farming by 2030. As part of the Soil to Table Action Plan, regulations are being developed for pesticides containing active ingredients derived from microorganisms. Compost teas, derived from composted materials, can provide a good alternative to diseases caused by plant pathogens, a property largely attributed to the presence of living microorganisms. A commercially available poultry manure, specially pelleted, has been shown in previous laboratory studies to benefit certain

crops. To find out the reasons behind this, its physico-chemical parameters were determined, and its microbiome was examined by metagenomic analysis. The third step of the studies is the identification of microorganisms that can be cultivated in the laboratory and subsequently selected for their potential antagonistic and plant growth-promoting effects. The pH and electrical conductivity (EC) tests of the compost tea demonstrated that while shaking at 25°C for 24 hours after pellet dissolution did not result in a significant change in pH, there was an increase in the EC value. A comprehensive analysis of the parameters of compost tea, including NO2-, NO3-, NH4+, PO43-, SO42-, and K+, was conducted to ascertain the chemical composition of the tea. The analysis revealed that the tea exhibited remarkably elevated concentrations of phosphate and sulfate. The phosphate solubility of compost tea is assumed to have a microbial composition capable of solubilizing inorganic phosphate. Shotgun sequencing was conducted on an Illumina platform with a 150-bp paired-end sequencing run. After assembly of metagenome contigs, quality control, and subsequent binning, BLASTn analysis was conducted against the NCBI Core Nucleotide Database to assign taxonomic identities to the contigs within the metagenome-assembled genomes (MAGs). The most common prokaryotic orders were Bacillales, Lactobacillales, Mycobacteriales, and Micrococcales. For the eukaryotic microorganisms, most genera were from Sordariomycetes, Eurotiomycetes, and Pichiomycetes. Among the microorganisms that could be isolated, bacteria were predominant, with Bacillus spp. and Paenibacillus spp. being the most common species. Examples include B. subtilis, B. velezensis, and P. polymyxa, which were also detectable in the metagenome analysis.

Evaluating Enterovirus Diversity among Symptomatic Patients in Hungary During and After Easing the COVID-19 Lockdown

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The COVID-19 pandemic led to widespread public health interventions that significantly affected the transmission of various pathogens, including enteroviruses (EVs). EVs exhibit considerable genetic diversity and can cause clinical manifestations ranging from mild illnesses to severe diseases. Our study aimed to evaluate the diversity of circulating EV types in Hungary and assess the impact of lockdown measures on EV prevalence based on testing clinical samples obtained from symptomatic patients. As part of the routine EV diagnosis, the National Reference Laboratory for Enteroviruses at the National Center for Public Health and Pharmacy conducts quantitative reverse transcription polymerase chain reaction (RT-qPCR) on clinical samples obtained from patients presenting with symptoms corresponding to EV infection. Positive samples are then subjected to virus isolation in cell culture and next-generation sequencing (NGS). Between January 2020 and December 2024, an overall number of 125 patients tested positive for EVs, mostly children under the age of 15 years. The most common symptoms were fever, hand-foot-mouth disease, encephalitis, and meningitis. The temporal distribution of EV-positive cases showed strong seasonality, with peaks in the summer and autumn months. The lowest number of confirmed cases occurred during the lockdown years, attributed to limited sample collection and reduced personal contacts. Following the easing of restrictions, the number of cases significantly increased, with the highest incidence observed in 2022. The distribution of EV genotypes shifted notably after easing the lockdowns. While only coxsackievirus (CV)A6 was detected during 2021, a broader range of genotypes emerged afterwards, including CVA10, CVA16, echovirus E9, and E11. Next-generation sequencing analysis revealed notable genotypic diversity, providing valuable insights into the evolution of EVs in Hungary and across Europe. Our findings underscore the importance of continued surveillance of enterovirus infections, particularly in the context of pandemic recovery, as the shifting EV genotype landscape may impact disease severity and spread, highlighting the need for adaptive public health responses.

Hepatitis A virus infections with genetically related strains in four neighboring countries

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Hepatitis A virus (HAV) is endemic in Hungary, but its incidence shows significant year-to-year fluctuations. The most recent outbreak occurred in 2022, mainly affecting the MSM population. In 2023 and the first half of 2024, only a small number of cases were reported; however, a resurgence began toward the end of 2024. Between January 1 and May 26, 2025, a total of 590 cases were reported, the majority from Budapest and Pest County. In parallel with Hungary, a substantial increase in hepatitis A cases has also been reported in Austria and the Czech Republic, while Slovakia has been experiencing a large-scale HAV outbreak since 2023. In none of the affected countries has a foodborne source been identified, and the infection appears to be spreading through person-to-person transmission. The detected viruses belong to subtype IB. The National Reference Laboratory for Hepatitis Viruses routinely performs genotyping of HAV strains from RNA-positive samples. In 2024–2025, the VP1/2A region —required for international comparison— was sequenced for 66 viral strains. Phylogenetic analysis was conducted by comparing Hungarian strains with sequences obtained from Slovakia and Austria. The detected viruses belonged to subtype IB, but differed from the strain responsible for the 2022 outbreak. The vast majority were identical to each other in the sequenced region, and three variants differed by only a single nucleotide position. Slovakian sequences from different years showed close genetic relatedness to the Hungarian strains, differing by 1–4 positions. In Austria, cases formed two distinct clusters: one identical to the dominant Hungarian strain, and the other matching one of the Slovakian variants. Notably, a sequence similar to the current strain was already detected in Hungary in 2022, but only in a single patient. Sequence data from the Czech Republic are not yet available. The data suggest a concurrent increase in HAV case numbers in several Central European countries, with slight demographic differences, but caused by HAV IB strains that are closely related genetically. Their similarity points to interconnected transmission chains. Our findings underline the importance of international collaboration, real-time molecular data analysis, and harmonized surveillance systems.

Examination of different staphylococcal species isolated from songbirds and their nestboxes

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Several staphylococcal species can be found in animals, which could serve as reservoirs for resistance and virulence genes for human pathogenic staphylococcal species, or can even cause human infections. Although birds often come in close contact with humans, few studies have investigated the prevalence of staphylococci in birds. The aim of the current study was to provide the first data from Hungary in this field. In October 2023, 28 samples were collected from empty nests in songbird nestboxes; and in May 2024, 20 samples were collected from nestlings. After incubation on appropriate culture plates, the suspected staphylococcus colonies were subcultured and stored at -80oC. Catalase-, tube coagulase, Clump- and oxidase tests were performed. Species identification was based on 16S rRNA sequencing and MALDI-TOF MS. The antibiotic susceptibility of the isolates

was determined by disk diffusion and gradient test. The presence of mecA, mecC, salA and lnuA resistance genes and tsst, eta, etb, sea, seb, sec toxin genes was detected by PCR. In total, 73 Staphylococcus strains were isolated, 45/28 from nestboxes and 28/20 from nestlings. The identified staphylococcal species were in ranking order: S. lentus (n=26), S. xylosus (n=19), S. sciuri (n=16), S. saprophyticus (n=6), S. succinus (n=3), S. hominis (n=1), S. vitulinus (n=1), S. warneri (n=1). S. aureus was not detected. S. sciuri, S. lentus and S. vitulinus (or sciuri group) have been reclassified into the Mammaliicoccus genus, these are frequently isolated from animals. The resistance rates of the isolates were the following: penicillin 86.3%, erythromycin 4.1%, clindamycin 57.5%, moxifloxacin 15.1%, tetracycline 6.8% and rifampicin 35.6%. High clindamycin resistance is not unusual in the sciuri group, and can be conferred by the salA (here: n=10) or lnuA (n=2) genes. On the other hand, rifampicin resistance was more prevalent among S. xylosus and S. saprophyticus. Three isolates were mecA positive: one strain each of S. hominis, S. sciuri and S. vitulinus, but only the S. hominis strain showed phenotypical resistance to cefoxitin. We could not detect mecC, neither toxin genes in this collection. In summary, high diversity of staphylococcal species were found among songbirds, many of them resistant to several antibiotics. The potential gene exchange between these species and S. aureus, during the relative frequent contact between these birds and humans, highlights the relevance of the 'One Health' concept.

Antifungal susceptibility of *Malassezia pachydermatis* isolates from companion animals and genomic insights into resistance mechanisms

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Malassezia pachydermatis is a lipophilic yeast frequently associated with otitis externa and dermatological disorders in companion animals. Reports of decreased susceptibility and treatment failure have raised concerns regarding the emergence of antifungal resistance, necessitating a comprehensive understanding of resistance mechanisms and reliable detection methods. This study aimed to evaluate the antifungal susceptibility of M. pachydermatis isolates from dogs and cats and to investigate the genomic determinants of reduced susceptibility. Susceptibility testing of 87 clinical isolates was performed using a modified CLSI broth microdilution method in Sabouraud dextrose broth supplemented with 1% Tween 80. The whole genome of ten representative isolates was sequenced and the genetic factors that are involved in drug resistance were investigated. Overall, ketoconazole, itraconazole, and terbinafine exhibited the highest efficacy (≤0.03 to 0.5 mg/L, ≤0.03 to 1 mg/L, and 0.03 to 1 mg/L respectively) while miconazole (0.5 to16 mg/L) and clotrimazole (2 to >32 mg/L) showed reduced activity against M. pachydermatis. Whole genome sequencing revealed single nucleotide polymorphisms (SNPs) in genes that play a key role in the ergosterol biosynthesis pathway, particularly in ERG11 and ERG1. While some specific amino acid substitutions (e.g., K446R in ERG11) were found only in isolates with elevated MIC values, no direct correlation with resistance could be unequivocally established. Genomic analyses also uncovered chromosomal mutations and heterozygosity of certain isolates, suggesting that complex, multifactorial mechanisms may drive the development of drug resistance. These findings highlight the importance of standardized susceptibility testing and further genomic investigations to promote effective antifungal therapy in both veterinary and human medicine.

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Enrichment of estrogen-degrading bacteria originated from dairy farms

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Global population growth, climate change, and agricultural practices – which consume approximately 70% of the world's freshwater – are intensifying water scarcity and degrading water quality through pollutants such as fertilisers, pesticides, antibiotics, and endocrine-disrupting chemicals (EDCs). Among EDCs, the large amount of estrogens originating from animal husbandry poses a severe ecological risk to aquatic ecosystems, including feminisation and infertility in wildlife. While physical and chemical removal methods exist, their cost and environmental impact drive interest in more sustainable approaches, such as bio-based treatments. Within the framework of the BioTreatED project, our goals are to isolate and identify microorganisms capable of reducing hormonal effects on a laboratory scale. Wastewater from intensive livestock farming was seasonally sampled from 3 locations in Hungary using standardised methods. From the samples, we were searching for bacterial strains that can utilise different estrogens (e.g., estrone (E1), 17β-estradiol (E2), or estriol (E3)) as their sole carbon source. Bacteria capable of growing in the presence of E1, E2, and E3 were selected using separate enrichment cultures and were subcultured onto R2A agar medium, followed by several purification steps. DNA was isolated from the pure cultures, and community DNA was also extracted from the enrichments. Until August 2025, 23 bacterial strains from the enrichments have been isolated, and their identification has begun using the 16S rRNA sequencing method. After species-level identification, the most promising candidates will be further analysed to characterise their estrogen-degrading capabilities.

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Characterization of the ZipV transcription factor in *Aspergillus fumigatus*

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The involvement of three bZIP transcription factor genes (Afu2g14350, Afu3g03230, Afu8g06260) in the virulence of the opportunistic fungal pathogen Aspergillus fumigatus Af293 was investigated. Only the deletion of the Afu3g03230 (zipV) gene resulted in attenuated virulence in a Galleria mellonella aspergillosis model. In surface cultures, the DzipV gene deletion mutant showed increased susceptibility to terc-butyl hydroperoxide compared to wild-type and complemented mutant strains, especially at low (1 g/L) glucose concentration. However, the gene deletion increased H2O2 and menadione-sodium bisulfite sensitivity only to a minimal extent and did not affect the nitrosative, osmotic, cell wall, heavy metal and iron limitation stress tolerance of the fungus. Re-evaluation of existing transcriptome data showed that the zipV gene was up-regulated by oxidative stress induced by H2O2 (on peptone), FeSO4 (both on peptone and glucose) and deferiprone iron chelator (on glucose) in submerged cultures. ZipV may influence virulence by contributing to protection against oxidative stress.

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The taxonomic diversity of prokaryotic communities in permafrost soil ecosystems in Patagonia, Chile

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The study of microorganisms inhabiting extreme environments offers a valuable opportunity to explore their adaptation mechanisms and ecological roles. Torres del Paine National Park, located in Patagonia (Chile), is part of the periglacial zone and is distinguished by the presence of permafrost soil and limited nutrient availability. The aim of the present study is to make a comparison between prokaryotic communities from two sampling sites that are located at different altitudes. Soil samples were collected from areas at 50 m and 1180 m above sea level, at depths of 10 cm and 35 cm. The Illumina MiSeq platform was used to reveal microbial diversity by amplifying the V3–V4 region of the 16S rRNA gene. At phyla level, Bacteroidota occurred at a higher rate in samples collected from near sea level than in those collected from mountainous areas, while Actinobacteriota, Gemmatimonadota, and Verrucomicrobiota were observed at lower rates. These variations can be explained by differences in the nutrient availability and moisture content of the sampling sites. The proportion of Chloroflexi was found to be remarkably high in samples obtained from mountainous regions. Within the domain of Archaea, the phylum Crenarchaeota exhibited a predominance, particularly in samples obtained from mountainous regions. This observation suggests a possible adaptation of these organisms to extremes of environmental stress. When examined at an order level threshold of 3%, the orders Acidobacteriales, Burkholderiales, and Gemmatimonadales were more prevalent in mountain samples, while representatives of the orders Chitinophagales and Rickettsiales were dominant in samples collected at lower altitudes. Members of the "Other" category occurred in high proportions, primarily in mountain samples, which indicates a higher degree of hidden diversity associated with increasing extremity. The NMDS analysis revealed two distinct groups between the sampling areas, with a significant difference (PERMANOVA, p-value=0.022), while the sampling depth had no significant effect on the microbial composition.
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Development of yield-enhancing bacterial soil and foliar fertilizer for crops that inhibits the spread of *Sclerotinia sclerotiorum*

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The use of vegetable oils has been shown a progressive development in recent decades, leading to a worldwide increase in cultivation. This trend, however also contributed to the spread of certain pathogenic fungi and the increase in the economic damage they caused. The innovation proposed here by BioFil Ltd. aims the creation of a complex soil and vegetation inoculant offering microbiological solution instead of synthetic fungicides in fighting the pathogenic fungus *Sclerotinia*

sclerotiorum and successfully promotes plant health and growth for oil and protein plants. The product contains only microbes isolated from domestic soil samples; therefore, its application raises no ecological risks, it is environment-friendly and sustainable. The innovation is aimed to be applied in intensive agricultural production, representing an environment-friendly, efficient alternative to chemical pest treatments and fertilization. The created product can also be used in organic farming. The bacterial inoculant has a positive quantitative effect on yields. The product is clearly a world novelty in its combined application (soil and both stock management) and dual effect (biofungicide and crop enhancer), and provides an environmentally conscious solution for the entire growing season. The EU's Green Deal initiative has set a target of reducing the use of chemical pesticides by 50% and increasing the share of organic farming to 25%, meaning that biological solutions will play an increasingly important role in plant protection and in agricultural production in the near future.
The project was implemented with the support of the European Union and co-financing of the Hungarian state, under Széchenyi Plan Plus program - GINOP PLUSZ-2.1.1-21 Fostering Corporate Research, Development and Innovation Activities.

In vivo therapeutic efficacy of synergistic antifungal peptide–drug combinations

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Due to the increasing spread of antifungal resistance, there is an urgent need for novel compounds with mechanisms distinct from those of conventional antifungals. Antifungal proteins/peptides (AFPs) and their de novo-designed synthetic derivatives have emerged as promising bioactive agents, effective alone or in combination with existing therapies. In our earlier studies, we demonstrated that synthetic peptides based on the evolutionarily conserved γ-core motif (GXC-X3-9-C, where X is any amino acid) of Eurotiomycetes-derived AFPs possess antifungal potential. In this study, we assessed γ-core derivatives of two predicted AFPs from Penicillium rubens (UniProt ID: B6GXZ8) and Aspergillus taichungensis (UniProt ID: A0A2J5HZT4), named Pr-γAFP and At-γAFP. Their in vitro antifungal activities were evaluated using broth microdilution against Aspergillus fumigatus, Botrytis cinerea, Candida albicans, Cladosporium herbarum, Fusarium subglutinans, and Saccharomyces cerevisiae. Synergistic interactions with conventional antifungals (amphotericin B [AMB], fluconazole [FLC], micafungin [MFG], and terbinafine [TRB]) were explored using checkerboard titration assays. Following toxicity assessment in the Galleria mellonella larval model, the in vivo therapeutic potential of synergistic combinations (Pr-γAFP with TRB against C. albicans, and At-γAFP with FLC against A. fumigatus) was further evaluated in the same model. Pr-γAFP and At-γAFP exhibited high in vitro activity against B. cinerea and S. cerevisiae, and F. subglutinans, respectively. Pr-γAFP+TRB showed synergy against C. albicans, while At-γAFP+FLC acted synergistically against A. fumigatus. Neither peptides nor antifungals, alone or in combination, reduced G. mellonella survival at tested doses. The combined administration of TRB and Pr-γAFP provided a superior therapeutic effect compared to their individual use and was capable of preventing infection development. At-γAFP exhibited moderate therapeutic potential as a monotherapy, which was further enhanced by the presence of FLC and resulted in prolonged larval survival. Overall, our results support the therapeutic potential of γ-core peptides as antifungal adjuvants.

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Evaluating the responses of soilborne fungal communities to artifical canopy gaps of different shapes and sizes in continuous-cover forestry systems

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Small-scale spatial dynamics driven by canopy gaps are well-known in plants, but have been scarcely studied in fungi. Gap-cutting is an integral component of continuous cover forestry and there are important, yet unanswered questions regarding how gap size and shape affect the soil biota. In this study, we compared soil fungal communities among plots with different gap size (small: 150 m2 vs. large: 300 m2) and shape (circular vs. elongated) in a 90-year-old sessile oak-hornbeam forest stand in Hungary. Soil samples were collected in March, 2022 in the Pilis Gap Experiment, that had been established in the winter of 2018/2019, with five treatments, including control, in six replicate blocks in a complete block design. ITS2 rDNA metabarcoding data were generated using Illumina MiSeq and were analyzed with dada2 in R, with taxonomic characterization done in usearch using the latest version of the UNITE database, with functional guild assignment using FungiTraits. We found no significant treatment effect on fungal community composition, with only the large circle treatment differing marginally from the control. Conversely, compositional difference among the blocks was significant for saprotrophs, but not ectomycorrhizal fungi, despite no apparent visual differences among the blocks at stand level. These results mirror the lack of treatment effect seen in understory vegetation published previously. Overall, despite formerly documented differences in abiotic factors among treatments (e.g., soil moisture, microclimate), most gap treatments were able to maintain fungal communities of the surrounding forest, including the dominance of site-specific factors in driving community structure. This means that gap-cutting is an appropriate tool to preserve forest fungal communities in continuous-cover forestry systems.

Compositional dynamics of forest plant pathogenic fungi in mosses, sedges, and oak trees along a disturbance gradient

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The effects of different forest management approaches on vegetation have been extensively studied. However, little is known about how forest management affects plant-associated fungal communities. As part of the long-term Pilis Forestry Systems Experiment in northern Hungary, we generated DNA metabarcoding data from leaves of three plant species that are common throughout the study area and represent different growth forms and three major clades of plants: Hypnum cupressiforme, Carex pilosa, and Quercus petraea. Our goal was to characterize plant pathogenic fungal communities in each host species and to compare their responses to forestry treatments. We sampled six replicate plots of clear-cutting (CC), gap-cutting (G), preparation cutting (P), retention tree group (R), and control (C). DNA sequences were identified and assigned to functional categories using the UNITE and FungalTraits databases, respectively. We found 810 plant pathogenic fungal genotypes, of which 261 occurred in mosses, 551 in sedges, and 410 in oak leaves. We observed compositional differences of plant pathogenic fungi among plant species, particularly between the moss and the vascular plants. Several fungal genera preferred a specific host and we identified several indicator species for each host. Treatments accounted for 21.55% of fungal compositional variance in mosses, 42.98% in

sedges and 34.75% in oak leaves. Compositional changes appeared to be proportional to the disturbance severity, with clear-cutting resulting in the greatest turnover of fungal species. Changes in plant pathogenic fungal communities correlated significantly with relative humidity, temperature measured at soil surface and at heights of 15 cm and 130 cm, and with vapor pressure deficit, indicating that a substantial effect of treatments on leaf fungal communities are mediated through changes in the microclimate. The observed compositional dynamics in leaf-associated fungal communities provide valuable insights into how different forest management activities shape plant pathogenic fungi, with possible implications for forest health.

The fungal genus Kalmusia is associated with grapevine trunk disease in Eger, Hungary

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Grapevine trunk diseases (GTDs) are devastating fungal infections affecting grapevines. Several syndromes and more than 300 fungal species are associated with these diseases. The pathogens infect the woody tissues of the plant, causing local necroses. Their phytotoxins can reach the annual parts of the plant and cause necrotic lesions on leaves and berries. It is widely believed that the pathogenesis of GTDs depends on the simultaneous action of several different fungal species, some of which are interchangeable, while a few "key" species—such as Phaeomoniella chlamydospora in the case of Esca disease—are indispensable. A metabarcoding-based study of naturally infected plants suggests that Kalmusia spp.—a group of lesser-known GTD pathogens—may play a crucial role in the development of foliar symptoms in Esca disease. In 2023, wood samples were collected by drilling from twenty symptomatic and ten asymptomatic grapevines in the vineyard of Eszterházy Károly Catholic University (Eger, Hungary). The first three leaves of symptomatic plants were also photographed. DNA was extracted from the samples and used in a metabarcoding-based investigation of the plants' endophytic fungal communities by sequencing the internal transcribed spacer (ITS) region using next-generation sequencing. The abundance of Kalmusia spp. showed a positive correlation ($p < 0.05$, Pearson test) with the severity of foliar symptoms (measured as the percentage of necrotized leaf area) in diseased plants. Additionally, the abundance of this fungal genus also correlated positively ($p < 0.05$, Pearson test) with the abundance of Phaeomoniella chlamydospora in asymptomatic plants. This potential relationship between Kalmusia spp. and Esca disease is surprising, as these fungi have only recently been described and are not typically considered major GTD pathogens. These results highlight the possible importance of Kalmusia spp. in the development of Esca disease. Despite the large number of related studies, this is the first report suggesting a role for Kalmusia spp. in Esca pathogenesis. We hypothesize that Kalmusia spp. may be replaceable in the disease complex, and that their function could potentially be carried out by other fungal taxa in different locations and/or vintages.

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Phylogenetic analysis of sequence variants of human papillomavirus 52

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High-risk human papillomavirus (HPV) types are associated with malignant lesions of the anogenital region. Members of Alphapapillomavirus 9 species play important role in the development of cervical cancer. The long control region (LCR) of HPVs is responsible for the regulation of viral replication and gene expression; and often used to describe intratype diversity and variant lineages. The sequence variants of the virus have different geographical distribution and variable oncogenic potential. Our research group has previously investigated the phylogenetic and functional differences of intratypic variants of some Alphapapillomavirus 9 types (HPV 16, HPV 31, 33). In the present study, we aim to identify the natural variants of HPV 52 in Hungary and to investigate the functional differences between them using LCR sequences. Exfoliated cell samples were collected from the uterine cervix of women with cytological and/or colposcopical abnormalities at the Department of Obstetrics and Gynaecology, University of Debrecen over a period of 15 years. After isolation of viral DNA, HPV genotyping was performed. DNA from HPV 52 positive cervix samples was amplified by PCR reaction with HPV 52 LCR specific primers. Multiple sequence alignment and phylogenetic analysis were carried out after sequencing the LCR variants. Fourteen specimens were selected for sequencing and phylogenetic analysis. From our samples, 7 variants, 10 single nucleotide changes and 2 deletions were identified. Five samples had the same sequence as the prototype HPV 52 variant. According to the constructed phylogenetic tree, our variants belong to lineage A, which is consistent with the international literature. In Europe, A1 is the most common sublineage (in about 90% of HPV 52 positive samples). Our variants showed a similar distribution, with 13 samples belonging to sublineage A1 and only 1 to sublineage A2. Most of our A1 variants showed 1 nucleotide difference from the prototype sequence, while in variant A2 we observed several point mutations and two deletions. In the future, we plan to perform functional analysis of the HPV 52 LCRs by transient transfection experiments and luciferase tests. Epidemiological and phylogenetical studies together with the functional analysis provide a basis for understanding the correlation between LCR polymorphism and the oncogenic potential of HPV 52 variants.

Probiotic origins and antifungal resistance in clinical *Saccharomyces cerevisiae* isolates from a Tertiary Clinical Center in Central Europe

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In recent years, Saccharomyces cerevisiae has received increasing attention as both a probiotic and a biotherapeutic agent. However, despite its beneficial effects —most notably the use of S. cerevisiae 'boulardii' in treating diarrheal diseases—it may act as an opportunistic pathogen, particularly in immunocompromised patients. Certain phylogenetic clades, including probiotic and baker's yeast strains, have been implicated in fungemia and invasive infections. Yet, the clinical relevance and pathogenic potential of these stains remain poorly understood. This study presents a comprehensive comparative analysis of 46 S. cerevisiae isolates collected over an eight-year period at the University of Debrecen Clinical Centre. Whole-genome sequencing, antifungal susceptibility testing (CLSI M27-A3), and plate-washing assays for invasiveness were performed. Multiplex PCR and phylogenomic

analysis identified 48% of isolates as probiotic in origin and 33% as baker's yeast. Most isolates belonged to clades such as Mosaic M3, Mixed origins 2, French dairy, Belgian beer 1 (diastatic), West European wine, and AU Wine 1. Notably, probiotic strains were overrepresented in the West European wine group. From a clinical perspective, 78% of isolates derived from adults and 57% from female patients. The respiratory tract was the most common source (60%), followed by the female genital tract (22%), and bloodstream (7%). Probiotic-derived isolates were significantly associated with pediatric cases (p=0.0321) and prior administration of probiotic therapy, particularly S. boulardii (p=0.0031). Baker's yeast isolates were less frequently associated with prolonged hospitalization (p=0.058), parenteral nutrition (p=0.055), and renal failure (p=0.0171), and exhibited significantly lower inflammatory markers (e.g., CRP, p=0.0121). Fluconazole resistance was observed in both probiotic and non-probiotic groups, though no statistically significant differences in treatment outcome or 30-day mortality were noted. In conclusion, clinical S. cerevisiae isolates often exhibit increased MICs to antifungals and belong predominantly to probiotic and baker's yeast lineages. Our findings highlight the potential for these isolates to cause opportunistic infections and underline the importance of genomic survaillance-based studies in healthcare settings.

Reversal of multidrug resistance using repurposed drugs as bacterial efflux pump inhibitors in *Escherichia coli* strains

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Antimicrobial resistance (AMR) is a growing global health threat caused by the overuse and misuse of antibiotics and the adaptive genetic mechanisms of bacteria. Among the mechanisms contributing to AMR, efflux pumps are key players in multidrug resistant (MDR) bacteria, such as Escherichia coli, by actively expelling antibiotics from bacterial cells. Drug repurposing has emerged as a promising strategy in the fight against infectious diseases. This approach aims to identify new therapeutic uses for already approved or clinically tested compounds. Psychiatric medications - such as antidepressants (e.g., SSRIs) and antipsychotics (e.g., phenothiazines) - are of particular interest due to their potential interactions with efflux pumps, which may confer antibacterial and resistance modifying properties. This study investigates the role of efflux pumps in antibiotic resistance in extended-spectrum β-lactamase (ESBL) producing E. coli strains and explores the potential of efflux pump inhibitors (EPIs) as therapeutic agents. The antibacterial and anti-biofilm activities of several EPIs, including promethazine (PMZ), thioridazine (TZ) were evaluated compared to reference EPIs, such as carbonyl cyanide m-chlorophenyl hydrazone (CCCP), and phenyl-arginine-β-naphthylamide (PAβN). The minimum inhibitory concentrations of antibiotics and EPIs was investigated by broth microdilution method. The efflux pump inhibitory activity of the EPIs was assessed using real-time fluorimetry, furthermore the anti-biofilm effect of the compounds was evaluated after crystal violet staining. The combined effect of ceftriaxone and PMZ was demonstrated using checkerboard assay on selected E. coli strains. Based on our findings, some repurposed compounds - particularly phenothiazine derivatives - demonstrated remarkable antibacterial activity. The results demonstrated that TZ exhibited antibacterial activity against certain E. coli strains. Anti-biofilm activity varied among the strains, with PMZ and TZ causing moderate reductions in biofilm formation. Notably, the checkerboard assay revealed no prominent synergy between ceftriaxone and PMZ. It can be concluded that drug repurposing as a promising approach to combat multidrug resistant bacterial infections.

Clarifying and detailing the roles of cfxA gene variants in β-lactam/ β-lactamase inhibitor combination resistance of *Bacteroides* isolates

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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: 50 B. fragilis group (BFG, 29 B. fragilis and 21 non-fragilis Bacteroides) isolates were selected whose amoxicillin/clavulanate (fixed ratio) MICs has been already recorded by agar dilution in an earlier antibiotic susceptibility study. These were retested by obtaining MICs by a fixed inhibitor concentration gradient method. We detected the cepA, cfxA, cfiA and pbbA genes by RT-PCR and sequenced the cfxA genes and their 5' regions. We performed conjugation experiments to transfer the resistance phenotype of some selected strains to a susceptible host. Results: One strain carried the cfiA and none the pbbA gene and all but one of the B. fragilis strains carried the cepA gene. The presence of the cfxA gene displayed the highest association with β-lactam/β-lactamase inhibitor combination resistance ($\chi 2$ AUG- BF $p<0.001$, NFB $p=0.149$, all $p<0.001$, AMC- BF $p=0.005$, NFB $p=0.004$, all $p<0.001$). The allele distribution of 31 sequenced cfxA-positive strains was as it follows: 11 cfxA2, 8 cfxA3, 2-2 cfxA4, cfxA5, cfxA10 and cfxA11 and 1-1 cfxA8 and cfxA9 where the heterogeneities mainly arouse at the 259 amino acid positions of the CfxAs. The upstream promoter regions of the cfxA genes seem to affect the resistance level as well as the cfxA gene types the substrate specificity. The β-lactam/β-lactamase combination resistance was transferable by conjugation only in one case of five studied strains. Conclusions: Here we revised and updated our previous results. As summary, we can say that the presence of the cfxA genes is responsible for β-lactam/β-lactamase inhibitor combination resistance in B. fragilis strains almost exclusively but among non-fragilis BFG strains to a lesser extent. Other affecting factors can be amino acid changes in CfxA or the activation mechanism of the cfxA genes. The resistance phenotype is transferable but not in every case.

Genetic and serological diversity of recent Fowl Adenovirus isolates

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Fowl Adenoviruses (FAdVs) are significant viral pathogens of chickens and are classified into five species (A–E) and further divided into 12 serotypes. Within FAdV-A and FAdV-B only one serotype is known (1 and 5, respectively), while FAdV-D contains four (2, 3, 9 and 11). For this study, a global set of FAdV-positive chicken samples were used and 97 isolates propagated on LMH cell line were selected for whole genome analysis. Based on partial sequence data they were previously identified as FAdV-A (n=32), FAdV-D (n=44) and one as FAdV-B. For whole genome sequencing DNA library was prepared from the extracted viral nucleic acid using Nextera XT DNA Library Preparation Kit, and sequencing was carried out on NextSeq 500 (Illumina) platform. Genomes were assembled and

aligned with Geneious Prime software. Genome-wide homology was assessed with the SimPlot++ tool, potential recombination events was detected by RDP5 software. To discover the serological diversity of the FAdV-D strains, cross-neutralization tests were performed. Sera were prepared in four-week-old SPF chickens subcutaneously vaccinated by experimental monovalent inactivated vaccines. Blood samples were collected 28 days post vaccination. The aliquoted sera were included in the virus neutralization (VN) test in two-fold dilutions. The 1:20 VN titer or above was considered positive. Based on the whole genome sequences we determined 11 genome types within the species FAdV-A, although it was known to be genetically conserved. Comparison of the studied FAdV B isolate with the few published complete genomes implies greater variation than previously thought; we found mosaicism in the recombination pattern. Most of the FAdV-D isolates (43 of 44) were clustered with serotype 2 and 11 strains. This was supported by cross-neutralization data. The detected high VN titers defined no distinct groups for serotype 2 and 11, but a common '2/11' serogroup, which is clearly separated from serotypes 3 and 9. A single FAdV-D isolate was clustered with serotype 3 sequences based on its genome sequence, and putative inter-serotype recombination events were found in the hexon and the fiber coding regions. The integration of genomic and phenotypic data underscores the importance of whole genome analysis in understanding FAdV epidemiology and virulence. Furthermore, the established serum panel and VN assay provide a valuable framework for justification of the phenotypic (serological) representation of genetic diversity.

Plant growth promoting bacteria from the rhizosphere of Szarvasi-1 energy grass

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Sustainable agriculture is one of the major challenge of the 21st century. The excessive use of synthetic fertilizers poses significant environmental risks, microbial-based biofertilizers could be promising alternatives. Plant growth-promoting bacteria (PGPB) are able to enhance the growth and resilience of plants. As part of a reclamation project, 76 bacterial strains were isolated and identified from the rhizosphere of Szarvasi-1 energy grass, which was sowed on dredged mud from lake Balaton. For the culturing, two selective media were used, one was specific for nitrogen fixing bacteria (NFb medium) and the other for phosphate solubilizing bacteria (Pikowskaya's medium). Bacteria were identified based on their 16S rRNA gene sequences. Each strain was tested for four PGPB traits: nitrogen fixation, phosphate solubilization, siderophore production, and indole-3-acetic acid production. Additionally, amplicon sequencing was performed (Illumina Novaseq 6000) to identify the bacterial community composition of the rizosphere. NGS analysis showed that Actinobacteriota and Pseudomonadota were found to be the most abundant phyla in the four rhizosphere samples, but representatives of the Chloroflexi, Gemmatimonadota, Bacteroidota, Firmicutes, and Myxococcota phyla were also present in all samples. 65% of the cultured strains are representatives of the Actinobacteriota phylum and 19% of the Pseudomonadota phylum, the rest of the strains are divided between Bacteroidota and Firmicutes phyla. The 76 isolated bacterial strains belonged to 27 different genera, of which Streptomyces, Promicromonospora and Cytobacillus appeared to be the most abundant. According to the results of the four PGPB tests, 82% of the strains grew on NFb medium (nitrogen free medium), 51% were able for phosphate solubilization (Pikowskaya's medium), 52% for siderophore production (CAS medium), and 32% for indole-3-acetic acid production (Salkowski reagent). 3 strains gave positive results for all the four tests: strain N51 Pseudoarthrobacter oxydans, strain P28 Rhodococcus erythropolis and strain P34 Rhodococcus fascians.

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Genomic Investigation of European *Acinetobacter baumannii* ST2 High-Risk Clone

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Multidrug-resistant Acinetobacter baumannii is a major concern in healthcare institutions worldwide. This study aimed to characterize A. baumannii isolates from Hungary and compare them to other European strains with a focus on virulence factors and antibiotic-resistance genes. Whole genome sequencing (WGS) was performed on 19 multidrug-resistant A. baumannii isolates from two hospitals in Budapest. Results were analyzed alongside data from 433 European A. baumannii isolates from the Pathogenwatch database. Among the 19 Hungarian isolates, 12 belonged to sequence type ST2 and 7 to ST636. Out of 12 ST2 isolates 11 carried blaOXA-23 and blaOXA-58 genes, while ST636 uniformly carried blaOXA-72. Core genome MLST revealed 3 subgroups within ST2: 10 strains of ST2 belonged to cgMLST906, one strain to cgMLST458, and one strain to cgMLST1320. Virulence gene profiles differed between ST2 and ST636, with OmpA present only in ST2, while many other virulence factors were present in all strains. Analysis of European isolates showed ST2 was the most prevalent (47.8%) which was widespread across Europe, followed by ST94 (12.2%) typical in the United Kingdom and ST636 (6.6%) mostly from Eastern Europe. This study provides insights into the genomic diversity of A. baumannii in Europe, highlighting the dominance of the ST2 high-risk clone. The analysis utilizing cgMLST offers a more precise identification in relation to antibiotic resistance genes and virulence factor genes and that shows the importance of the application of WGS for the studying of MDR A. baumannii clones, and that have implications for surveillance and control of this important nosocomial pathogen.

The role of the yap1 gene in the stress response of *Aspergillus fumigatus*

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Aspergillus fumigatus is an opportunistic fungal pathogen causing aspergillosis. While it generally poses little risk to individuals with a healthy immune system, it can lead to severe infections in immunocompromised patients, such as those with cancer, AIDS, or undergoing organ transplantation. One of the most serious conditions it causes is invasive aspergillosis, which is associated with high mortality rates and limited treatment options, making it a significant clinical challenge. Its pathogenicity is based in part on resistance to host immune defenses, in particular oxidative stress. This study investigated the role of the Yap1 transcription factor in A. fumigatus stress responses. Surface cultures of wild-type and Δyap1 gene-deleted strains were exposed to oxidative, osmotic, cell wall and heavy metal stresses. The mutant showed significantly reduced growth under tert-butyl hydroperoxide, menadione, hydrogen peroxide and diamide-elicited oxidative stress compared to the wild type. Deletion of yap1 did not affect growth under osmotic (sodium chloride, potassium chloride and sorbitol), cell wall (Congo red) or heavy metal (cadmium chloride) stress, indicating that its function is highly specific for oxidative stress tolerance. These results indicate that Yap1 plays a key role in the defense mechanism of A. fumigatus against oxidative stress. A deeper understanding of Yap1 function can highlight its potential as a target for novel antifungal therapies.

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Foodborne Viruses: Emerging Challenges in Food Safety

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Foodborne viruses represent a significant public health concern and are increasingly recognized as major contributors to outbreaks of gastroenteritis and hepatitis worldwide. Among the most important pathogens are noroviruses, rotaviruses, hepatitis A virus (HAV), and hepatitis E virus (HEV), which together account for the majority of documented foodborne viral infections. Unlike bacterial foodborne pathogens, viruses cannot multiply in food; however, their remarkable stability in diverse environmental conditions allows efficient transmission via contaminated food, water and food handlers. Recent epidemiological studies highlight the dominance of norovirus as the leading cause of non-bacterial gastroenteritis, responsible for substantial morbidity and economic burden. Rotaviruses, although primarily associated with pediatric infections, remain an important cause of severe gastroenteritis in young children and continue to pose a foodborne transmission risk in settings with inadequate hygiene. HAV continues to cause large-scale outbreaks, often linked to contaminated shellfish, frozen berries, or inadequately washed products. Meanwhile, HEV, long considered geographically restricted, has emerged as a global zoonotic and foodborne pathogen, frequently associated with undercooked pork and game meat. Foodborne viruses cannot be detected using classical virological methods, as cultivation of most of them is still not possible in cell culture. Consequently, molecular methods are indispensable for their identification, yet they remain limited in assessing viral infectivity. The gold standard remains real-time PCR; however, in recent years, digital PCR and next-generation sequencing technologies have rapidly advanced, raising great expectations among food virologists. Still, since viral loads in food are often very low, this poses an additional challenge. The absence of effective antiviral treatments for most foodborne viruses underscores the importance of preventive strategies. These include rigorous hygiene in primary production, effective sanitation of irrigation and processing water, compliance with good manufacturing practices, and strict monitoring of food handlers. Special attention must be given to the implications of globalization, climate change, and changing dietary habits on the dynamics of viral foodborne transmission. Understanding these complexities is crucial for developing integrated approaches to mitigate the risk of viral foodborne infections.

Long-term survival of *Francisella tularensis subsp. holarctica* LVS within amoebae

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Francisella tularensis is a gram-negative bacterium, the causative agent of the zoonosis tularaemia. It is able to survive in environmental niches, including amoebae. As free-living protozoa, Acanthamoeba spp. represent a potential environmental reservoir that may contribute to the long-

term persistence of the pathogen in the form of more resistant cysts. The aim of this study was to investigate whether F. tularensis subsp. holarctica LVS can survive long-term in cysts of the amoeba Acanthamoeba castellanii, and whether it can be recovered from the cysts in such a case. A. castellanii was infected with F. tularensis subsp. holarctica LVS with a multiplicity of infection (MOI) of 50. Monitoring of the presence and number of bacteria was initially performed using the CFU method, which included differentiation of cysts into trophozoites, lysis of amoeboid cells and plating of tenfold dilutions on chocolate agar at 6, 24, 48 and 72 hours and at 7, 30 and 60 days post-infection. The presence of bacteria in the cysts was further analysed using confocal microscopy. Using this method, the bacteria could be successfully isolated up to a maximum of 72 hours after infection. However, confocal microscopy confirmed the presence of bacteria in the cysts up to the maximum analysed period of 60 days post-infection. Various in vitro methods for recovery of the bacteria were tested, including recovery in immortalised murine macrophages and macrophages from human whole blood monocytes, as well as in vivo recovery by intranasal inoculation of the cysts into C57BL/6 mice. None of the tested in vitro methods led to bacterial recovery. In vivo experiments, however, led to the isolation of bacteria from the lungs, liver and spleen of mice, and they could be successfully recovered from the cysts 60 days after infection. Our study has shown that F. tularensis subsp. holarctica LVS can survive in A. castellanii cysts up to the maximum studied period of 60 days post-infection. The successful recovery in mice confirms the existence of the VBNC form and the potential importance of amoebae as reservoirs and mediators of transmission in the natural environment.

PREVALENCE AND GENETIC DIVERSITY OF VIRUSES INFECTING RED RASPBERRIES IN SERBIA

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Red raspberry (Rubus idaeus L.) is the most important berry fruit in Serbian agriculture. Rubus species are hosts of more than 30 viruses and virus-like diseases, and less than 10 viruses were confirmed infecting raspberries in Serbia. From 2015 to 2024, a number of 193 samples were collected from 86 orchards from different locations in raspberry-growing areas in the country. The most of collected samples showed different leaf symptoms, as blotch, yellows, twisting, and chlorotic mottle, while others were asymptomatic. Samples were tested for the presence of six viruses: raspberry leaf blotch emaravirus (Emaravirus idaeobati, RLBV), Rubus yellow net virus (Badnavirus reterubi, RYNV), black raspberry necrosis virus (Sadwavirus rubi, BRNV), raspberry leaf mottle virus (Closterovirus macularubi, RLMV), raspberry vein chlorosis virus (RVCV), and raspberry latent virus (RpLV). The analysis was performed with RT-PCR and PCR assays using virus-specific primers. The most prevalent virus that was confirmed in analyzed samples was RLBV (49.7%), followed by RLMV (17.6%), RYNV (15%), BRNV (9.3%), and RpLV (6.7%). RVCV was not detected in any of the analyzed samples. The frequency of mixed infection was very high (87.5%), including two to four viruses. Based on the results, virus distribution maps were created. Selected isolates of detected viruses were partially sequenced, and their phylogenetic relationship was evaluated. The sequences of the Serbian RLBV isolates showed 92.8−100% nucleotide (nt) sequence identity, which is similar to that of isolates from other countries. When compared, Serbian RYNV isolates showed high (97.4–100%) nt sequence identity. BRNV isolates share 94.2–99.7% identity, but they are quite divergent in comparison with isolates from Europe and North America (80.0–82.2% nt identity). Very high nt sequence identity was detected in RLMV isolates (99.5–100%). All Serbian RpLV isolates have identical sequences among themselves and with the isolate from North America. As presented, the highest rate of infection was recorded with RLBV which is present in several European countries, its economic impact was confirmed, but it is still not monitored in any country.

Boosting anaerobic lignocellulose utilization via syntrophic interactions

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Lignocellulose-based biomass is one of the most abundantly available forms of renewable raw materials, offering significant potential for the sustainable production of energy and chemical feedstock. Our goal is to establish an efficient lignocellulose-degrading microbial community that remains stable in an artificial environment with the help of tight syntrophic interactions. We examined the degradation efficiency of consortium members originating from the rumen — anaerobic fungi (AGF) from the fibrous phase, methanogenic archaea (MA), and bacteria (BAC) — when grown on straw, using SEM, HPLC, and metagenomic methods. SEM images vividly illustrated microbial proliferation as well as close cell-to-cell interactions between AGF, MA, and BAC members. Metagenomic sequencing enabled the monitoring of dynamic changes in the community composition, while HPLC analysis revealed the composition of the products formed during the degradation process. As previous studies have confirmed the effectiveness of biomass pretreatment, we also applied biological pretreatment in our experimental system. The anaerobic fungus–methanogen (AGF-MA) and anaerobic fungus–methanogen–bacterium (AGF-MA-BAC) consortia produced significant amounts of methane when supplied with hydrogen. The AGF-MA-BAC consortium produced 27% more methane compared to AGF-MA alone, and 50–60% higher methane yields in the optimal microbial combinations. Following the pretreatment, biogas fermentation tests were carried out. The AGF-MA-BAC consortium again achieved the highest methane yield, further supporting our hypothesis that this microbial minimal community promoted the structural degradation of plant biomass and also enabled superior methane production during the process. The methane produced, as a main component of biogas, can be directly utilized as a renewable energy source. This synergistic system therefore offers a promising solution for the energy-efficient processing and utilization of lignocellulose-rich biomass and waste.

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SUSTAINABLE YIELD IMPROVEMENT IN LEGUMES USING BENEFICIAL MICROORGANISMS

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Legumes play a vital role in ecosystem nutrient cycling, particularly nitrogen, because they can form symbiotic relationships with nitrogen-fixing rhizobia and mycorrhizal fungi. These endosymbioses not only provide legumes with essential nitrogen, phosphorus, and micronutrients, but also enrich the soil. In agricultural systems, this leads to additional benefits for subsequent crops in both intercropping and crop rotation practices. However, like other crops, legumes are increasingly challenged by climate change, which can severely reduce yields. Stress-tolerant rhizobia and biostimulant soil microbes offer promising approaches to mitigate the negative impacts of environmental stress factors. To enhance the productivity of legumes, projects have focused on isolating soil microbes from legume roots and the rhizosphere. These isolates are being characterized

for their plant-growth-promoting activity, drought tolerance, and synergistic effects with rhizobia. In parallel, microbiome analyses of mutant plants with root defects are being conducted to understand better how root development influences microbial community composition. The findings are expected to support the development of climate-resilient legume cropping systems and provide strategies for sustainable agroecosystems.

Eliminating manganese sensitivity and boosting citric acid production in *Aspergillus niger* through transporters manipulation

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Citric acid, a widely used organic compound, is overwhelmingly produced by submerged fermentation of carbohydrate-rich substrates using Aspergillus niger, a filamentous fungus recognized for its high yield, metabolic robustness, and ability to tolerate low pH conditions. Citric acid overflow requires a unique combination of unusual conditions: high concentrations of H+, dissolved oxygen and initial carbon source; as well as Mn(II) ion concentrations below 5 ppb. Manganese at above such minuscule concentrations is easily introduced by other culture ingredients, and must be removed from the medium through costly and laborious processes to support high-yield citric acid production. Manganese uptake into cells is facilitated by specific transporters. In A. niger, the transporter DmtA plays a critical role in manganese acquisition from the medium, which subsequently influences biomass formation, morphology and conidiospore germination. As well, abolition of dmtA expression, as shown in ΔdmtA, increases biomass-specific citric acid production even in environment with abundant manganese. Secretion of citric acid into the culture broth occurs mainly via the citrate exporter CexA. The upregulation of cexA under manganese limitation is a major reason why manganese deficiency is necessary for high-yield citric acid production. In this study, we demonstrate that an A. niger ΔdmtA ΔglaA::cexA mutant strain, where cexA is overexpressed under the control of the strong glucoamylase (glaA) promoter in a dmtA-deficient background, can accumulate citric acid to levels even higher than those of the parent (i.e., unmutated transporters) strain under manganese-deficient conditions, despite the presence of abundant manganese. Furthermore, the fermentation time–a critical parameter in industrial processes–is significantly reduced, making this strain an attractive cell factory for industrial applications.

THE ANALYSIS AND IDENTIFICATION OF POTENTIAL PROBIOTIC CANDIDATES FROM COMMON CARP (CYPRINUS CARPIO) GUT AND GAMETES

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Common carp (Cyprinus carpio) is a key species in Hungarian pond aquaculture; therefore, environmentally friendly and sustainable methods to improve reproductive performance are of great interest. One possible approach is the development of targeted probiotics in fish feed. This study analysed the initial gut and gamete microbiomes of common carp using traditional and molecular techniques to select microorganisms with potential probiotic activity for future commercial use. Sampling and microbiological analyses were conducted on 15 male and 15 female carp from a Hungarian fish farm. Microbial culturing was performed on Tryptone Soy Agar (TSA) and selective De Man-Rogosa-Sharpe (MRS) media. DNA from gut and gamete samples was extracted using

commercial kits, and microbial communities were profiled by Illumina amplicon sequencing of the 16S rRNA gene (V3–V4 regions). Data were processed with the DADA2 pipeline, and Amplicon Sequence Variants were annotated using the Silva 138.1 database. Aeromonas dominated the gut microbiome of both sexes, while Cetobacterium was more abundant in male fish. Members of Erysipelotrichaceae, Desulfovibrionaceae, and Brevinema were abundant in the microbiome of some individuals. Only one ovarian fluid sample was successfully analysed by next-generation sequencing due to the low microbial DNA yield of gametes. In this sample, we found an overwhelming dominance of the Mycoplasma genus. Gamete microbiome analysis relying on cultivation combined with MALDI-TOF MS identified Aeromonas, Pseudomonas, Acinetobacter, Lactobacillus, and Shewanella species. In total, 125 potential probiotic isolates were obtained; the 20 most promising (Lactococcus, Lactobacillus, Liquorilactobacillus) were characterized following EFSA recommendations. Technological parameters (optimal temperature, medium, pH), safety, and effectiveness were assessed through phenotypic and molecular tests. Based on preliminary results, Lactiplantibacillus plantarum (TÉT43) was selected as the most promising candidate for probiotic feed development and was prepared for in vivo testing.

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Consortium-based microbial inoculants for climate-resilient agriculture in temperate cropping systems

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Agricultural production in temperate regions faces increasing challenges due to rising average temperatures, uneven precipitation leading to desertification, and the emergence of novel plant pathogens driven by climate change. At the same time, widespread use of chemical pesticides and synthetic fertilizers has led to food chain contamination, eutrophication, and nitrate pollution. In response, there is growing interest in alternative, environmentally sustainable soil management approaches, particularly those leveraging the plant-beneficial effects of soil microorganisms. Our research focuses on the development of complex, consortium-based microbial soil inoculants designed to mitigate the adverse impacts of climate change on key crops, including soybeans, maize, sunflowers, and walnuts. These formulations combine multiple beneficial microbial strains with complementary functions, aiming to overcome one of the major limitations of microbial soil treatments: inconsistent field performance. The selected strains are screened for climate resilience, drought tolerance enhancement, nutrient mobilization capacity, plant growth promotion, and biocontrol potential against soilborne pathogens. The prototype inoculants are currently undergoing greenhouse and open-field validation. Notably, they are expected to reduce synthetic fertilizer inputs, enhance plant resilience under drought conditions, and contribute to the rehabilitation of degraded or contaminated soils by improving soil structure and nutrient availability. In addition to traditional criteria (yield improvement, disease resistance, and growth stimulation), our development strategy emphasizes the selection of microbial consortia adapted to local, variable climatic conditions, thereby supporting more reliable and sustainable crop production. Preliminary results from field trials on maize, sunflower, soybean, and walnut plantations will serve as illustrative case studies.

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West Nile virus in Hungary: A phylogenetic study based on integrated mosquito and human surveillance

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West Nile virus (WNV), a widespread mosquito-borne pathogen, is endemic to many parts of Europe, including Hungary, a known hotspot for locally acquired human infections. At least nine distinct lineages of the virus have been identified, with lineages 1a and 2 responsible for the majority of human and animal infections. During the 2024 transmission season (May to November), 7,410 Culex spp. mosquitoes were collected from seven trapping sites in and around Budapest. The mosquitoes were grouped into 474 pools and tested for the presence of WNV. Human clinical samples (n=42) were tested using Sanger sequencing for real-time surveillance and reporting to the ECDC (European Centre for Disease Prevention and Control). In addition, next-generation sequencing (NGS) was performed on 46 WNV-positive samples from 30 patients and one mosquito pool using Illumina platforms in collaboration with the Bernhard Nocht Institute for Tropical Medicine. Preliminary data indicate the predominance of WNV lineage 2, specifically the Balkan sub-cluster, during the 2024 transmission season. Only one human-derived WNV sequence from Budapest belonged to the Central European cluster. Based on complete genome sequences—primarily from neuroinvasive cases collected between 2015 and 2023—the geographic distribution of the Balkan sub-cluster was previously limited to southeastern Hungary. This study presents the first genomic evidence of a northward expansion of the Balkan sub-cluster into the Budapest region. A more detailed phylogeographic analysis is currently underway to confirm these findings. NV circulation in nature is maintained through a complex enzootic cycle involving mosquitoes and avian hosts. Genomic surveillance of WNV strains from both human and mosquito samples provides insights into the emergence and spread of new or existing variants. Such surveillance enhances our understanding of virus transmission patterns and the potential role of viral genetics and evolution in driving morbidity and mortality.

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Validation of a naovel membrane-filtration based method for the detection of *E. coli* and coliform bacteria in recreatonal water

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Recreational water are natural water bodies like oceans, lakes, and rivers, as well as human-made systems like artificial lakes and ponds, that are used for leisure and sporting activities. The role of these waters will be more and more appreciated, especially during extreme warm summer seasons. However, proper water quality is essential to protect the health of bathers. According to the

2006/7/EC Bathing Water Directive, the quality of the bathing waters based on the four-year dataset of feacal indicator bacteria (E. coli and Enterococcus). Examination of these bacterial parameters from natural bathing water is usually not easy, especially for the E. coli parameter. Although some different standards are available, not all of them are applicable on the basis of the legislation's provisions. On the other hand, at the useable membran filtration method (ISO 9308-1) the large number of background microbiota makes the evaluation difficult the evaluation, while the MPN method (ISO 9308-3) requires different consumables and a lot of attention. Directive does not permit the use of Colilert method (ISO 9308-2), despite this method gives result within one day, but quite expensive and needs special equipments. In the 2025 bathing season the watermicrobiology laboratory of the National Center for Public Health and Pharmacy tested a new membrane filtration method for define E. coli count from natural waters. All in all 18 bathing waters and other surface waters were chosen from Pest County. Different volume (100, 50, and 10 ml) of the samples were filtered paralelly on mix cellulose esther membrane with 0.45 µm pore size. Membranes were placed on tryptone bile-X-glucuronide (TBX) agar. TBX agar plates were put at 36 °C for 4 h to 5 h to resuscitate stressed cells, followed by incubation at 44 °C for 21 h. After incubation, E. coli colonies appeared blue or blue green color, based on their β-D-glucuronidase positive properties. Samples were processed with the well known membrane filtration, microplate and also Colilert methods beside the new, TBX-based method. Although the statistical analyses are ongoing, preliminary data show that the results obtained with the different methods are similar, so the TBX-based method could be a fast, cheap and easy way to define E. coli count from recreation waters.

FUNCTIONAL CHARACTERIZATION OF SPORE SURFACE PROTEINS IN *MUCOR LUSITANICUS*: CORRELATIONS WITH VIABILITY AND PATHOGENICITY

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The Mucorales order includes highly resilient fungi responsible for mucormycosis, a lethal and emerging infection, and the third most common angio-invasive fungal disease. CotH proteins play a central role not only in pathogenicity but also in regulating spore germination and proper spore structure formation. These spore surface proteins are crucial for adhesion, colonization, and infection. In this study, we characterized Mucor lusitanicus cotH-disrupted mutants to assess cell wall alterations. Fluorescent staining and TEM were used to monitor structural changes. To assess cell viability, the XTT assay and FUN1 staining were used, while apoptosis and necrosis quantification was applied to distinguish between different forms of cell death. CotH mutants showed diverse responses to cell wall stressors, with specific gene deletions leading to modifications in the inner spore coat and affecting fungal growth and sporulation. Moreover, our results highlight that several CotH proteins are indispensable for infection. The loss of CotH6 protein displayed abnormal sporangial morphology and decreased sporulation capacity. TEM analysis of the cotH9 mutant revealed morphological hallmarks of programmed cell death, including pronounced cytoplasmic disorganization, vacuolization, and structural fragmentation, indicating severe intracellular dysfunction and highlighting the critical role of CotH9 in both spore and hyphal viability. In Drosophila melanogaster model the pathogenicity were reduced in the ΔcotH9 and ΔcotH12 strain, suggesting a host-specific virulence function. The absence of CotH12 protein caused atypical septation and spore wall formation, observed through TEM. A deeper understanding of how this opportunistic pathogen utilizes CotH proteins not only advances our knowledge of fungal development and pathogenicity but may also inform the development of novel diagnostic and therapeutic strategies. Given that the outcome of mucormycosis heavily relies on early diagnosis which is requiring advanced imaging and

molecular tools, for that very reason the CotH protein family is emerging as a promising focus in both molecular diagnostics and as a potential target for antifungal therapy.
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Extracellular proline production of plant growth promoting rhizobacteria in high salt containing media

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Under osmotic stress (for example high salinity or drought) one crucial survival method for plants and bacteria is the accumulation of osmolytes, and one of the most essential osmolyte for many plants and bacteria is proline. As a proteinogenic, zwitterionic amino acid, proline can substitute water and help living organisms to maintain their osmotic potential, and turgor pressure when they face lack of water. The intracellular raise of proline concentration is well documented mainly in plants; however, it is less evident how bacteria modify extracellular proline content under high salinity circumstances. In this study our aim was to assess proline release and production of plant growth promoting rhizobacterial strains cultivated in high salt containing media. We used five halotolerant strains of PGPRs: Peribacillus frigoritolerans ES85, Kocuria rosea S225 and S273 and Bacillus tequilensis ES8 and ES9. For screening we cultivated the strains in complex media: Tripton Soy Broth, Biofil Kocuria Medium, and Biofil Bacillus Medium with several salt concentrations (NaCl) to assess whether these strains were able to accumulate extracellular free proline under osmotic stress. After the positive results we inoculated our bacterial strains into minimal salt media to measure their extracellular proline production. We used Agrobacter Minimal Medium for K. rosea strains, modified Ratnaningrum Minimal Medium for B. subtilis strains, and as minimal salt medium hasn't yet been established for the Peribacillus genera, we have developed Peribacillus Minimal Medium for P. frigoritolerans ES85. For proline detection we applied ninhydrin colour reaction and spectrophotometric measurement. All five strains elevated the free proline level of composite media in presence of high salt concentrations (starting from 4-6 m/v% NaCl) compared to the control settings. Moreover, both of K. rosea and B. tequilensis strains and showed elevating proline production in minimal salt medium at higher salt concentrations.
The project was carried out with the support of the National Research, Development and Innovation Fund under the ACCELERATOR Programme 2020-1.1.5.

Microbiologically Influenced Corrosion – Insights from Field-Relevant Experiments

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Microbiologically influenced corrosion (MIC) poses significant challenges across industrial sectors such as oil and gas, cooling systems, and utilities. Defined as corrosion influenced by the presence and/or activity of microorganisms, MIC necessitates an interdisciplinary approach bridging microbiology, chemistry, and materials science. Despite advancements in understanding microbial mechanisms, progress is often limited by disciplinary silos and challenges in translating laboratory

results to field conditions. This presentation offers microbiologists a detailed overview of MIC, advocating for experimental designs that closely mimic real-world scenarios. Our laboratory study utilized carbon steel coupons exposed to drinking water and corrosion products from a severely corroded distribution system. We integrated molecular microbiological techniques (ATP biomass measurement, shotgun metagenomics) with chemical assessments (redox potential, pH, and metal concentrations) and advanced imaging methods (SEM-EDX, 3D optical microscopy). We identified an iron-reducing bacterium that has not been previously studied in drinking water distribution systems, which may have implications for corrosion processes. In light of this discovery, our research highlights the need to simulate field conditions, the effectiveness of shotgun metagenomics in MIC studies, and underscores the necessity of a microbiological approach in experimental designs, utilizing a multiple lines of evidence (MLOE) strategy in laboratory studies. Further investigation into these microbial dynamics will optimize corrosion management strategies in industrial settings.

BbAtfA plays an important role in the growth, stress response and conidiation of the insect pathogenic fungus *Beauveria bassiana*

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Beauveria bassiana is a well-studied and important entomopathogenic fungus, as it is often used in plant protection as a biological control agent against various insect pests. Orthologs of the Schizosaccharomyces pombe Atf1 and Aspergillus nidulans AtfA bZIP-type transcription factors are involved in coordinating various processes in the vegetative mycelium of filamentous fungi, such as sexual and asexual development, defence against environmental stress, and regulation of secondary metabolism. They are also important in the virulence of plant and human pathogenic fungi and are also thought to play an important role in the virulence of insect pathogenic fungi, such as B. bassiana. The roles of the atf1-atfA orthologous BfatfA gene in B. bassiana are not yet fully elucidated, therefore, in this study we investigated the phenotypic consequences of BbatfA deletion under different culture conditions. Based on agar plate stress sensitivity assays, the ΔBbatfA mutant strain showed reduced growth on stress-free control agar medium compared to the wild-type strain, a phenotype previously described for A. nidulans and F. verticilloides. The gene deletion mutant was more tolerant to Congo Red, which is used to test cell wall integrity stress, furthermore, this strain was osmophilic under osmotic stress conditions induced by NaCl and sorbitol. The mutant also showed enhanced sporulation under hyperosmotic conditions. These results suggest that the BbatfA gene negatively regulates vegetative growth and conidiation in high-osmolarity environments, while it is required for optimal growth under unstressed culture conditions. The aim of our further experiments is to determine whether the stress tolerance of conidia is affected by the presence or absence of osmotic stress during sporulation.

Hantavirus infections in Hungary between 2018 and 2025

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Hantaviruses are rodent-borne zoonoses distributed worldwide. Two species of Old-World hantaviruses are circulating in Hungary: the Dobrava-Belgrade virus, which causes hemorrhagic fever with renal syndrome (HFRS), and the Puumala virus, which causes a milder form of HFRS, nephropathia epidemica (NE). Wild rodent species are the natural host and principal reservoir of the hantaviruses. Human infections occur via direct or indirect contact with infected rodent excreta, mainly through inhalation of aerosolized virus particles. Between 2018 and May 2025, the Hungarian National Reference Laboratory for Viral Zoonoses received 1080 test requests for hantaviruses. Due to the short viremic period, hantaviruses are primarily diagnosed by serological methods using indirect immunofluorescence assay and immunoblot assay. Detection of viral nucleic acid using real-time or nested RT-PCR is only possible from samples collected in the early stages of the disease. Nested PCR amplicons were sequenced using the Sanger method. Phylogenetic analysis based on the 345-nucleotide-long region of the hantavirus S segment nucleocapsid gene. Between 2018 and May 2025, 50 cases of hantavirus infection were confirmed in Hungary. Of these, approximately 60% were caused by the Dobrava-Belgrade virus and 40% by the Puumala virus. 82% of the confirmed cases were males, suggesting that hantavirus infection is significantly more frequent among males than females. Twenty patients out of the 50 serologically-confirmed cases were tested by PCR method, and 27 samples were found to be PCR positive. Puumala virus RNA was detected in 13 samples (12 serum samples and 1 EDTA-treated whole blood) of 12 patients, and Dobrava-Belgrade virus RNA was detected in 14 samples (5 serum samples, 6 EDTA-treated whole blood, and 3 urine samples) of 8 patients. In 3 cases of Dobrava-Belgrade virus infection, the viral RNA was detectable only from the EDTA-treated whole blood sample, but not from the serum. Four Puumala and 6 Dobrava-Belgrade nested PCR amplicons were successfully sequenced. Our sequences showed a close phylogenetic relationship with virus strains from Germany, Slovakia, Turkey, and the Balkan countries. Only a limited number of hantavirus infections are diagnosed in Hungary annually. Serum and EDTA-treated whole blood samples collected early stage of the infection are the most suitable for nucleic acid detection of hantaviruses.

A cloning strategy to express aflatoxin B1 degrading genes of *Rhodococcus erythropolis* NI86/21 in R. jostii

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To identify aflatoxin (AFB1) degrading genes of R. erythropolis NI86/21 we carried out two experiments: 1. we identified 48 up-regulated proteins by proteome analysis of control and AFB1 induced NI86/21 cells, 2. seven proteins showed homology to AFB1 degrading proteins of Mycobacterium smegmatis in an in silico analysis. We selected 5 – 5 gene/gene products to clone and express in R. jostii, a weak AFB1 degrading strain. To prove the concept that AFB1 degrading proteins can increase the AFB1 degrading capacity of a weak degrader, the gene of MSMEG_5998 (an F420 dependent reductase) of M. smegmatis was synthesysed together with a methanol inducible promoter and cloned into the BglII restriction sites of the pFAJ2574 vector. Competent R. jostii cells were transformed, and two transformants were selected and screened for elevated AFB1 degradation in a thin layer chromatography experiment. Both transformants showed dramatic

increase of AFB1 degrading activity, approving our concept, and opening avenues to clone, express and validate putative AFB1 degrading genes. For biochemical characterisation of AFB1 degrading proteins we are planning to use Strep-tag affinity purification, since we found quite a number of proteins - like cation transporters, multicopper oxidases, siderophores and the hydrogenase nickel incorporation protein HypB - with HGHG motifs/repeats, which resemble to metal binding histidine repeats, in most of the Rhodococcus strains. We carried out affinity purification experiments in E. coli expression system with a recombinant protein encoding for the MGSHGHGHGHGHGHGP motif of the HG717_10955 cation transporter, as affinity tag, the Ta0895 protein of Thermoplasma acidophilum serving as solubilisation tag, the ENLYFQG cleavage site, and a short sequence of the pRSFDuet vector multi cloning site starting from the NdeI site until the stop codon down stream of the S-tag. This protein could bind to Ni-, Co-, and Zn-NTA affinity beads indicating the role of the HGHG motif in metal binding, and forcasted unwanted protein contamination upon His-tag purification of proteins expressed with 6xHis tag in Rhodococcus strains.
The study was supported by the National Research, Development and Innovation Office (Hungary) – Grant number [K142686].

DETECTION OF A PELARSPOVIRUS SEQUENCE IN DOGWOOD (CORNUS SP.) EXHIBITING VIRUS-LIKE SYMPTOMS

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In 2003 a dogwood (Cornus sp.) plant exhibiting ringspots and line patterns along veins on leaves was first observed in south-western Slovenia. Later, in 2009 similar symptoms were observed on dogwood plant in central Slovenia. A leaf sample of this plant was collected and tested by ELISA for several nepoviruses, but all tests were negative. Since no viruses were found in this plant by serological method, the plant was sampled again in 2021 to be examined by molecular approach. Total RNA was extracted and subjected to high-throughput sequencing (HTS) using an Illumina platform (Macrogen, Korea). As part of the Euphresco project VIRNOTree ("Surveillance for viruses and other non-culturable pathogens in forestry tree species") the data were further analysed. A subsample of reads was de novo assembled and compared against local plant virus database. The analysis showed numerous contigs mapping to the members of the genus Pelarspovirus. Current efforts are focused on completing the viral genome by analysing the whole dataset, and designing specific primers for virus detection. Additional dogwood samples with or without symptoms were collected from central Slovenia to assess the connection between virus infection and symptom presence and to explore its geographical distribution in Slovenia. These results highlight the value of HTS as a diagnostic tool when conventional assays fail to identify the causal agent inducing the symptoms on plants.
The work was funded by the Ministry of Agriculture, Forestry and Food of Slovenia, and Slovenian Research and Innovation Agency (ARIS Program P4-0072 and P4-0431).

Investigation of a recombinant β-galactosidase from *Rhizomucor pusillus*

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Zygomycetes fungi, especially members of Mucoromycota, represent a diverse group of filamentous fungi; many of which are saprotrophic and have industrial, biotechnological, and medical significance. β-Galactosidase (lactase) hydrolyzes β-glycosidic bonds in β-galactosides and is widely used in the food industry to reduce the lactose sugar mass in dairy products and to process whey, a by-product of cheese production. Due to the prevalence of lactose intolerance, there is a growing demand for novel β-galactosidases with desirable biochemical properties and cost-effective production systems. The Mucoromycota fungi can be exploited for enzyme production; however, their β-galactosidase activity and the molecular and functional features of the corresponding genes are less studied. In this study, genome analysis of the thermophilic Rhizomucor pusillus SZMC 11025 revealed three putative β-galactosidase coding genes that show a high similarity to Trichoderma atroviride β-galactosidase. The predicted proteins differed in size, sequence length, and isoelectric point. A conserved domain characteristic of the glycoside hydrolase family 35, which may be responsible for activity, was identified in all predicted proteins. Coding cDNA sequences were isolated and cloned into Komagataella phaffii expression vectors, and the recombinant enzymes were produced and purified via affinity chromatography. Purification yield was superior to those of most fungal β-galactosidase expressed in heterologous systems to date. The purified β-galactosidase exhibited optimal activity around 60 °C and pH 5.8-7.0. To our knowledge, this is the first heterologous production of a β-galactosidase from a zygomycetous fungus. The targeted expression system allowed the isolation of the enzyme with sufficient purity for detailed biochemical characterization. Future work will focus on optimizing the heterologous production for higher enzyme yield as well as further characterization of the biochemical properties of the purified enzyme. The R. pusillus β-galactosidase can be a promising and cost-effective catalyst for food industry applications, including lactose removal from dairy products and the synthesis of galacto-oligosaccharide (GOS) and lactulose-derived GOS (OsLu) prebiotic sugars.

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Synergistic antifungal activity of caspofungin and posaconazole against *Candida auris* biofilms

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The World Health Organization (WHO) had designated Candida auris as a critical public health threat due to its multidrug resistance to antifungal agents, high mortality rate, andits ability to cause nosocomial outbreaks. A matter of serious concern is that conventional antifungal monotherapies is frequently ineffective against biofilm-associated C. aurisinfection, highlighting the need for more effective strategies. Combination therapies have demonstratedimproved efficacy and specificityand may also slow the development of antifungal resistance. Therefore, this study aimed to evaluate the in vitro and in vivoefficacy of caspofungin (CAS) and posaconazole (POSA) in combination against sessile C. auris isolates from the South Asian clade and to characterize the associated transcriptomic responses. The CAS with POSA combination resulted in a significant reduction in median minimum

inhibitory concentrations (4–32-fold for CAS and 8–64-fold for POSA) compared to monotherapies. Synergistic interactions were observed in all isolates tested, with fractional inhibitory concentration indices ranging from 0.078 to 0.31. In line with the in vitro findings, synergistic interactions were confirmed by in vivo experiments. The fungal kidney burden decreases were three log volumes in mice treated with combination of 1 mg/kg/daycaspofungin and 1.5 mg/kg/day posaconazole. To investigate the gene expression changes in response to combination treatment, 612 genes showed increased expression and 465 genes were downregulated relative to the untreated control biofilms (fold change >1.5 or <–1.5). Notably, genes involved in biofilm dispersion, oxidative stress response, iron metabolism, and glycolysis were downregulated, whereas those associated with cell wall organization or biogenesis, osmotic stress response, calcium signaling, drug efflux, biofilm formation, and the biosynthesis of ergosterol and chitin were upregulated. These findings demonstrate the potent synergistic activity of CAS and POSA against C. auris biofilms and provide insight into C. auris antifungal therapeutic responses, thereby contributing to the development of more effective antifungal treatment strategies.

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Species Distribution and Risk Factors of Non-albicans Candidaemia in a Hungarian Tertiary Care Centre

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Candida bloodstream infection remains a life-threatening condition associated with high mortality, particularly in intensive care units. In recent years, the global incidence of non-albicans Candida species has risen significantly, accompanied by an epidemiological shift from Candida albicans to non-albicans species as the predominant pathogens in candidaemia. This study aimed to investigate the predisposing factors and clinical outcomes of patients with candidaemia caused by non-albicans species. A total of 75 patients (mean age: 56 years; 52% female) treated at the University of Debrecen Clinical Centre between January 2024 and August 2025 were included. The most common underlying conditions were the presence of a central venous catheter (83%), urinary catheter (86%), and renal failure (68%). The distribution of Candida species isolated from blood cultures was as follows: C. albicans (41%), C. parapsilosis (21%), C. glabrata (13%), rare Candida species (13%), C. tropicalis (9%), and C. krusei (3%). The overall 30-day mortality rate was 57%, with lower mortality observed in C. albicans infections (52%) compared to non-albicans species (61%). Corticosteroid therapy was significantly associated with C. albicans infections (odds ratio [OR]: 4.93; 95% confidence interval [CI]: 1.52–16.01; p = 0.01). Similarly, prior fluconazole treatment was more common among C. albicans cases (OR: 4.29; 95% CI: 1.31–14.5; p = 0.021), suggesting that exposure to corticosteroids and fluconazole may be important risk factors for C. albicans candidaemia. The median planktonic minimum inhibitory concentrations (pMICs) of the isolates ranged as follows: fluconazole, 0.125–32 mg/L; amphotericin B, 0.015–2 mg/L; anidulafungin, 0.008–2 mg/L; caspofungin, 0.032–4 mg/L; and micafungin, 0.032–4 mg/L. Biofilm production assessed by the crystal violet assay revealed that C. tropicalis isolates formed significantly greater biofilm biomass compared to C. albicans and C. glabrata (p < 0.001–0.01). This comprehensive analysis enhances the understanding of candidaemia in healthcare settings and may contribute to improved clinical management and reduced patient mortality.

Clinical and Banana-Associated *Fusarium musae* Under Antifungal Pressure

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Fusarium musae, part of the Fusarium fujikuroi species complex (FFSC), is emerging as an opportunistic human pathogen. While it is primarily known for causing postharvest crown rot in bananas, it has also been isolated from clinical cases involving keratitis, onychomycosis, and invasive infections in immunocompromised patients. Contaminated bananas may carry Fusarium conidia, contributing to household exposure. This study aimed to assess biodiversity and the in vitro antifungal susceptibility profiles of clinical (N = 6) and banana-associated environmental (N = 19) FFSC isolates. Clinical isolates were obtained from various human samples (e.g., sputum, sinus secretions), while environmental strains originated from banana fruits and peels. Species-level identification of Fusarium isolates was performed using MALDI-TOF mass spectrometry for proteomic profiling, and further confirmed through phylogenetic analysis of partial TEF1-α and RPB2 gene sequences, interpreted via the curated FUSARIOID-ID database (https://www.fusarium.org/). Antifungal susceptibility testing was performed for all isolates according to CLSI guidelines, with in vitro susceptibility assessed against amphotericin B, voriconazole, posaconazole, isavuconazole, and two novel agents olorofim and manogepix. All confirmed isolates belonged to the F. musae clade within the FFSC. MIC values for F. musae strains showed high variability across both clinical and environmental sources. Azole resistance was prominent, with voriconazole and isavuconazole MICs frequently ≥16 mg/L, and posaconazole MICs ranging from 0.5 to >16 mg/L. Amphotericin B exhibited moderate activity (MICs 2–16 mg/L), while olorofim and manogepix showed promising activity in a subset of isolates (olorofim MICs as low as 0.5 mg/L, though often >16 mg/L; manogepix MICs consistently >16 mg/L). Notably, a mild Eagle effect was observed in several isolates, characterized by a paradoxical increase in growth at higher olorofim concentrations (e.g., 16 mg/L), while reduced growth was noted at lower concentrations (1–2 mg/L). This phenomenon suggests a complex response of Fusarium musae to olorofim and calls for further investigation to better understand its mechanism and clinical relevance. F. musae was frequently isolated from both clinical and banana-associated environmental samples, supporting the hypothesis that contaminated bananas may act as a vehicle for human exposure and possible infection.

Biochar-based carriers for microbial inoculants in sustainable agriculture: A study on viability and persistence

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Biochar is a promising carrier for microbial inoculants in sustainable agriculture. In order to achieve a comprehensive understanding of the full potential of biochar, it is imperative to investigate the manner in which it interacts with diverse microbial species over extended periods of time. However, the long-term viability of different strains across diverse biochar types is not fully understood. This study evaluated the survival of three free-living microbes (Bacillus amyloliquefaciens, Streptomyces spp., and Trichoderma spp.) on five types of biochar derived from sunflower husk, wood chips, green waste, pelletized green waste, and a wood–zeolite mixture (75:25). Prior to microbial inoculation,

the surface properties of the biochars were evaluated using BET analysis. Brunauer–Emmett–Teller (BET) analysis is a technique used to measure the specific surface area of porous materials by nitrogen gas adsorption, providing insight into their porosity and adsorption capacity. Sunflower-based biochars exhibited low surface areas (1.6–3.0 m^2/g), while wood chips and green waste (both pelletized and non-pelletized) showed moderate values (11–66 m^2/g). Wood–zeolite samples displayed relatively high surface areas (~146 m^2/g) and pore volumes (>0.1 cm^3/g), likely due to zeolite's microporosity. A total of 45 biochar–microbe combinations were prepared under sterile conditions in 3 replicates. Viability was tracked weekly over a ten-week period from late February to early May using the Most Probable Number (MPN) method. A long-term follow-up assessment was conducted in late June. Bacillus and Trichoderma maintained viability throughout the test period, especially on sunflower and wood–zeolite carriers. Streptomyces declined sharply after week 3 and was undetectable by week 5. Pelletized green waste supported the lowest viability across strains. At the post-assessment nearly two months later, only Bacillus and Trichoderma remained viable, while other strains showed no activity, consistent with prior trends. The findings demonstrate that biochar type and physicochemical characteristics strongly influence microbial survival. These results support the targeted selection of carrier materials to improve the durability of biochar-based microbial inoculants in sustainable agricultural systems.

Agricultural use of spent mushroom compost

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In white button mushroom (Agaricus bisporus) cultivation, casing material, primarily peat-based—covering compost colonized by mycelium—plays an important role in fruiting body formation and ensures high water retention. Currently, the supply of casing material for mushroom cultivation is a serious problem, as peat mines are being depleted. Furthermore, peat extraction is a destructive industry, as peatlands—the world's largest terrestrial carbon sink - are one of the most important tools for mitigating climate change. Therefore, developing new and healthy casing material alternatives to avoid environmental damage and pollution is a pressing issue. Good quality casing material contains a variety of microorganisms. Our goal was to test spent mushroom compost as a healthy alternative to casing material. Spent mushroom compost contains both beneficial and harmful bacteria (e.g., Bacillus, Pseudomonas, Microbacterium, Alcaligenes) and fungi (e.g., Trichoderma, Hypomyces, Fusarium, Lecanicillium, Mortierella, Rhodotorula). Many species of the genera Bacillus, Pseudomonas, Phanerochaete, and Rhodotorula exhibit beneficial properties. Microorganisms (bacteria and fungi) were isolated and identified from spent mushroom compost samples taken during a natural recomposting process and from additional champignon growing materials. The isolates were identified, characterized (ecophysiological activity, enzyme activity, indoleacetic acid and siderophore production) and a consortium was established to help the natural recomposting process, which resulted in a peat-like material full of nutrients. Mushroom cultivation trials in bags revealed, that mixed with peat in a 90:10 ratio, the recomposted spent mushroom compost is excellent for use as casing, furthermore, it also has potential as plant growing medium, therefore it has the potential to become a succesful example of circular agriculture.
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Microbial Diversity and Iron Metabolism in Corrosion-Associated Communities: Metagenomic Insights for Water Infrastructure Monitoring

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Microbial processes contribute signific antly to corrosion in drinking water systems, impacting infrastructure and water quality. In the context of integrated catchment management and the EU Water Framework Directive, understanding these microbial dynamics is essential for modernizing water system monitoring. Carbon steel coupons were incubated in tap water microcosms under nutrient-amended and unamended conditions. Biofil m and bulk water samples were collected across three experimental batches. After shotgun metagenomic sequencing, we performed taxonomic and functional profil ing. We assessed alpha diversity metrics across a range of Hill numbers (q = 0-8), including species richness, Shannon diversity, Simpson diversity, and Pielou's evenness. To account for sequencing depth variability, rarefaction-based estimation and ANCOVA modeling were applied. Microbial diversity varied signific antly by sample type and nutrient condition. Bulk water without nutrients and water control showed higher richness and Shannon diversity than biofil ms without nutrients. Nutrient addition decreased diversity in bulk water, driven by dominance of opportunistic taxa, but increased diversity in biofil ms, suggesting enhanced niche partitioning. Full Hill number profil es revealed subtle dynamics, with nutrient-added biofil ms showing crossovers in evenness at mid-q values (0.6-1.5), indicating resilience to perturbations through maintained balance among mid-abundant species. ANCOVA confir med that sample type and nutrient status explained ~80% of diversity variation, with Shannon diversity unaffe cted by sequencing depth. Functional profil ing using FeGenie identifie d genes related to iron storage, siderophore synthesis, heme transport, and iron regulation. Nutrient availability, rather than spatial niche, was the main driver of gene abundance patteerns. This study demonstrates the utility of metagenomics in characterizing corrosion-associated microbial communities. By linking diversity and iron metabolism to environmental conditions, the fin dings support the development of microbial indicators for water infrastructure monitoring within integrated water management systems.
This research was carried out within the framework of the Széchenyi Plan Plus program with the support of the RRF-2.3.1-21-2022-00008 project. We acknowledge the Digital Government Development and Project Management Ltd. for awarding us access to the Komondor HPC facility based in Hungary.

Impact of arbuscular mycorrhizal fungal inoculation on powdery mildew infected tomato plants

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.Arbuscular mycorrhizal fungi (AMF) enhance plant resilience to abiotic stresses like drought, salinity, and heavy metals, and can influence biotic stress responses by improving water and nutrient uptake and regulating defence pathways. While their abiotic roles are well known, little is understood about their interactions with obligate biotrophic pathogens. Erysiphe neolycopersici obligate biotrophic fungus poses a serious global threat to both greenhouse and field production, spreading rapidly via airborne spores and causing reduced photosynthesis, stunted growth, and yield losses. Management is complicated by its obligate lifestyle, limited chemical control options, and increasing fungicide

resistance, underscoring the need for alternative strategies such as AMF-based approaches. Our study examines how Funneliformis mosseae colonization shapes the defence responses of tomato (Solanum lycopersicum L.) during infection by Erysiphe neolycopersici, the causal agent of tomato powdery mildew. Tomato 'Moneymaker' 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 powdery mildew infection to simulate biotic stress conditions. After a four-week growth period, plants were harvested, and physiological parameters together with disease severity were quantified. Furthermore, the activity of the reactive oxygen species scavenging enzyme glutathione-S-transferase was measured, and the relative gene expression of the phenylalanine ammonia-lyase gene – an essential component of the salicylic acid-mediated defence pathway against biotrophic pathogens – was evaluated. Our preliminary results could contribute to a better understanding of plant-microbe interactions with potential applications in sustainable crop management.

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High-throughput viability assay for studying soil and host preference patterns of dark septate endophytes

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Dark septate endopytes (DSEs) are a taxonomically diverse group of fungi that can colonize plant roots and form microsclerotia within them. DSEs have both ecological and agricultural importance. A high-troughput microplate viability assay – using a fluorescent dye to evaluate cell activity – was adapted to filamentous fungi to study the preference of DSEs to host plants and dissolved organic matter (DOM) content. This method is faster, easier, more precise than dish based cultivations. Isolates of two important DSEs, Periconia sp. and Setophoma terrestris, from Martonvásár and Fülöpháza, were used in the experiments. Maize and wheat from Martonvásár were grown under sterile conditions. Roots were harvested, dried, crushed, and dissolved in distilled and sterilesed by filtration. To model DOM, water extractable organic matter (WEOM) extraction was performed from sandy soil from Fülöpháza. The dried WEOM was dissolved in destilled water then autoclaved. A five-step serial dilution was prepared from both plant and WEOM stock solutions. Diluted WEOM stocks were used to complement PDB medium, whereas plant filtrates were applied without PDB. The stocks were inoculated with fragmented hyphae and a viability assay was performed. Fungal growth was detected under both conditions. Both maize and wheat root filtrates can be used in microplate assays. A higher signal was observed with maize filtrate, and isolates derived from maize showed a preference for maize root filtrate. This phenomenon was not observed for isolates from wheat. In the case of WEOM, the highest concentration inhibited the growth of all isolates, while at lower concentrations the two species responded differently. It is possible to use this promising high-troughput method to investigate in vitro the preference of DSEs to host plant and soils with varying DOM concentrations.

Comparison of the core microbiome and indicator species of vineyards and surrounding Rosaceae fruit species

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The plant microbiome, especially its fungal component, plays a crucial role in host plant health, stress tolerance, and development. Comparing fungal associations in cultivated and wild plant species can provide insights into the diversity and ecological functions of plant-associated fungi and its relevance for sustainable agriculture. In this study, we examined and compared fungal communities found in the woody tissues of grapevine (Vitis vinifera), pear (Pyrus communis), apricot (Prunus armeniaca), dogrose (Rosa canina), and blackthorn (Prunus spinosa) within a heterogeneous agricultural landscape in the Eger wine region (Hungary). Sampling was conducted during March, June, and September of 2021 and 2022, in vineyards and orchards embedded in a mosaic of shrubland. Fungal ITS2 rDNA sequences were generated using Illumina MiSeq and processed with DADA2 in R. Taxonomic assignments were made using USEARCH with the UNITE reference database, and functional group classification was based on the FungalTraits database. Core genera were defined as those present in at least 80% of samples per plant species. Statistically significant indicator fungal species, exclusive to or predominantly associated with a single host, were identified for each host plant. We identified 58 core fungal genera across the five plant species. The most common genera—Alternaria, Aureobasidium, Buckleyzyma, Filobasidium, Taphrina, Symmetrospora, and Xenodidymella—were found in at least four host species, with Alternaria occurring in all. Several of these are known endophytes or facultative pathogens. Aureobasidium, notable for its biocontrol potential, was also widely distributed among samples. Blackthorn had the highest number of indicator species among the wild species, whereas grapevine ranked highest among the cultivated ones. Our findings underscore the ecological value of wild plants in agroecosystems and the potential use of fungal community analysis in agroecological monitoring.

Characteristics of Nitric Oxide Dismutase (Nod) genes in paddy soils under different climatic gradients in China

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Nitric oxide dismutase (Nod), a key enzyme mitigating nitrous oxide (N_2O) emissions by catalyzing nitric oxide disproportionation into nitrogen and oxygen, remains poorly characterized in agriculturally critical systems. Here, we investigated the biogeography, environmental drivers, and assembly mechanisms of nod-harboring microbial communities across 40 paddy soil samples spanning tropical to mid-temperate climatic zones in China. Quantitative PCR revealed the highest nod gene abundance in subtropical soils (1.22×10^8 copies·g^{-1} dry soil), correlating positively with total nitrogen ($r = 0.36$, $p < 0.05$) and soil moisture ($r = 0.37$, $p < 0.05$). PacBio sequencing identified 192 operational taxonomic units (OTUs) clustered at 90% similarity, dominated by the NC10 phylum (67.3% of OTUs). Community composition exhibited distinct climatic zonation: Candidatus Methylomirabilis (NC10) dominated tropical soils (32.7%), Bradyrhizobium prevailed in subtropical regions (28.9%), and Pseudomonas/Streptomyces characterized temperate zones. Community structure was primarily shaped by pH ($R^2 = 0.41$), mean annual temperature ($R^2 = 0.38$), and

precipitation (R^2 = 0.35). Stochastic processes dominated community assembly (accounting for 75.7-97.8% of β-diversity turnover), with stochastic drift increasing significantly with latitude (33.3% in subtropical to 71.8% in mid-temperate zones), driven primarily by dispersal limitation and ecological drift. Despite representing only 0.02-0.03% of total bacterial communities (based on 16S rRNA gene abundance), nod gene abundance constituted 15-54% of canonical denitrification genes (nirS/nirK), suggesting a disproportionate N_2O mitigation potential. Variance partitioning attributed only 24.3% of community variation to measured environmental factors, leaving 75.7% unexplained - a "missing fraction" potentially implicating unmeasured biotic interactions. These findings establish nod communities as climate-sensitive regulators of N_2O flux and advocate for region-specific mitigation strategies: optimizing water management in subtropical zones to sustain NC10 activity and amending temperate soils to enhance reactor-derived taxa. This study provides the first continental-scale framework for harnessing Nod-mediated N_2O reduction in agroecosystems, advancing climate-smart rice cultivation.

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Respiratory Viral Infections in Hospitalized Children in Croatia: A Pre-Pandemic Study

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Acute respiratory infections (ARIs) are the most common infections in children, with approximately 80% caused by viruses. To describe the epidemiological characteristics of viral ARIs in children from the northern part of mainland Croatia, we conducted a prospective study as part of a Croatian Science Foundation project. Over a four-year period (2017–2021), we enrolled 957 patients under 18 years of age admitted to the Clinic for Children's Diseases Zagreb or the General Hospital Karlovac with symptoms of ARI. The median age of tested children was 1.86 years; 540 were male and 417 female. For virological diagnosis, nasopharyngeal and pharyngeal swabs were collected, and multiplex PCR was performed. One or more viruses were detected in 739 (77.2%) patients: a single virus in 496 (67.1%) cases and multiple viruses in 243 (32.8%) cases. The most frequently detected virus was rhinovirus (RV) (343; 35.8%), followed by respiratory syncytial virus (RSV) types A and B (162; 16.9%), adenovirus (145; 15.2%), parainfluenza virus types 1–4 (101; 10.6%), bocavirus (73; 7.6%), influenza virus types A and B (69; 7.2%), enterovirus (61; 6.4%), coronaviruses OC43 and 229E/NL63 (54; 5.6%), and metapneumovirus (30; 3.1%). Across all age groups, RV was the most common virus detected. The second most frequent virus varied by age group: RSV A/B in children under one year (90/311; 28.9%), adenovirus in children aged one to three years (66/296; 22.3%), and influenza A/B in children over five years (26/232; 11.2%). The incidence of all viruses was similar in the 2017/18, 2018/19, and 2019/20 seasons but declined significantly in 2020/21. To identify clinical species of the most common virus, rhinovirus, further analysis of RV-positive samples was performed. Sequence analysis of a 395 bp fragment of the 5'UTR region revealed 69 distinct genotypes: RV-C (47.4%), RV-A (44.7%), and RV-B (7.9%). No differences in clinical presentation were observed among RV species. Overall, more than half of hospitalized children with RV infection (55.8%) presented with lower respiratory tract infection. Notably, RV was detected in 10 of 18 children who developed acute respiratory distress syndrome during the study period, six of whom had RV as a monoinfection. These findings highlight the predominance of RV in pediatric ARIs and its association with severe disease.

Impacts of microplastic pollution on soil and rhizosphere microbiota

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Terrestrial ecosystems are exposed to extensive pollution with plastics and microplastics (MP), with direct agricultural inputs and unintentional release especially impacting arable soils. A profound understanding of the interactions of MP with physical, chemical, and microbial factors in agricultural soils is critical to predict impacts on soil functions and fertility. Also in the rhizosphere of crop plants, the impacts of MP pollution are still poorly understood. Our ongoing research, embedded within the Collaborative Research Center 1357 "Microplastics" of the University of Bayreuth (www.sfb-mikroplastik.uni-bayreuth.de), aims to elaborate comprehensive insights into the response of prokaryotic and fungal soil microbiota to MP pollution. We employ environmental sampling, bench-scale microcosms, and controlled plot-scale field experiments to determine MP-induced alterations in soil biogeochemical parameters and processes, as well as changes in the structural and functional composition of soil- and plant-associated microbiota. In unplanted soil microcosms, we show that soil microbes tend to be more clearly responsive to biodegradable vs. conventional plastics, but that effects strongly vary with soil type. Next, maize (Zea mays) and strawberries (Fragaria × ananassa) were cultivated in soils amended with different types of conventional (LDPE, PET, PS) or biodegradable (PBAT) MP. The results reveal a dominating influence of plant hosts on prokaryotic soil microbiomes, largely overruling direct impacts of MP across soil compartments. However, significant abundance changes were observed for several typically plant-relevant microbial taxa, including polymer degraders, PGPRs and also arbuscular mycorrhiza. Effects observed for different plant species, soil compartments, and plastic types were mostly distinct, which clearly complicates the more general conclusions we would like to make. Our ongoing work aims to unravel the metabolic potential and functional context of microbial responses to plastic pollution. This is an important contribution to the development of more sustainable management schemes and remediation strategies for plastics as a still largely uncharted risk for soils and agroecosystems.

Upgrading spent oyster mushroom substrate for potential soil bioremediation: Effects of post-harvest treatments

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Oyster mushroom (Pleurotus ostreatus) is one of the most common edible mushrooms cultivated on an industrial scale. At the end of production, spent mushroom substrate (SMS) remains in high amounts as organic waste. As P. ostreatus is a white rot fungus, it produces ligninolytic enzymes like laccase, to break down lignin present in wood, but they are capable of degrading chemical compounds too, for example soil pollutants. Our general aim is to use SMS as a bioremediation agent to help the degradation of soil pollutants like polycyclic aromatic hydrocarbons and mineral oils. As P. ostreatus naturally grows on wooden material and decomposes lignocellulose-based substrates during its large-scale production too, it is difficult to find conditions where it can effectively grow in soil and help the degradation of soil contaminants. The objective of the experiment was to test if post-harvest treatments can help the regrowth of mycelia in SMS and possibly enhance the production of ligninolytic enzymes, thereby upgrading the SMS to be more effective in soil bioremediation. In the treatments, SMS was mixed with (1) spent coffee grounds and (2) a mixture of wood chips and sawdust, compared to a control treatment (3), where SMS was only mixed and

incubated for one month. We measured 6 types of enzymatic activities weekly and tracked the changes in P. ostreatus mycelial mass compared to the total fungal and bacterial biomass in the substrate by qPCR. In the second part of the experiment, we mixed the control and supplemented SMS samples to soil polluted with mineral oils, and we have been monitoring enzymatic activity with two more soil indicator enzymes, mycelial growth and the degradation of the pollutants over a two-month incubation period. According to our results, during the first month (post-harvest treatment) the examined enzymes showed higher activity after mixing, followed by a gradual decline week by week. In most cases, the enzyme activity was not significantly higher in the supplemented samples than in the control treatment. The results of the second part of the experiment show that after adding the SMS samples to soil, most enzymatic activities dropped to nearly zero however, the two examined soil indicator enzymes, acid-phosphatase and dehydrogenases increased week by week. Data for contaminant concentration and qPCR will be presented during the lecture.
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Alternative respiratory pathways in *Aspergillus niger*: their impact on citric acid fermentation

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Alternative oxidase (AOX) provides an alternative respiratory pathway that functions alongside the main electron transport chain in plants, fungi, and certain animal cells. While AOX has been extensively studied in plants, interest in its role in fungi is steadily growing. Citric acid production is predominantly carried out by Aspergillus niger, which can convert carbon sources into citric acid with an exceptionally high yield of up to 95%. This efficient conversion involves the rapid catabolism of large amounts of glucose, leading to ATP accumulation, which in turn allosterically inhibits key irreversible steps of glycolysis. AOX plays a pivotal role in maintaining high glycolytic flux by decoupling NADH oxidation from ATP synthesis through oxidative phosphorylation. This bypass relieves feedback inhibition and promotes sustained glucose metabolism. The activity of AOX is associated with increased aeration, which typically enhances reactive oxygen species (ROS) formation; however, AOX also contributes to cellular defense by mitigating ROS accumulation. Aspergillus niger possesses two aox genes. Phylogenetic analysis indicates that one of them, aoxA, is ubiquitously conserved across species. The second gene, aoxB, likely originated from horizontal gene transfer, possibly from a species within the Onygenales order, outside the Aspergillaceae family. Several studies have investigated aoxA in the context of citric acid production, using overexpression and deletion mutants to examine its function and regulation. However, until now, no study has simultaneously addressed the roles of both aox genes. Our group has analyzed citric acid production in single (aoxA, aoxB) and double deletion mutants, providing new insights into how the two AOX isoforms influence the physiology and productivity of A. niger.

Surveillance of swine coronaviruses in Hungary

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Monitoring the occurrence and genomic variations of coronaviruses (CoVs) is inevitable for laying the foundation of effective containment strategies. The aim of this study was surveillance of swine coronaviruses in Hungary. One hundred and twenty-one specimens (84 oral fluid samples, one sampling rope per pen; 34 pools of nasal swabs, 4–5 swine per pool of 162 animals; 3 processing fluid samples, retrieved during castration) were collected from healthy animals in pig farms in Hungary, and were tested with a pan-CoV RT-PCR system developed in our laboratory. CoV sequences were detected in 15 specimens originating from four settlements. Five sequences were shown high identity with porcine hemagglutinating encephalomyelitis virus, while ten with porcine respiratory CoV/ transmissible gastroenteritis virus sequences. Since CoVs, including porcine CoVs, are prone to recombination, whole genome analysis could be performed to reveal more details on their evolution.

Novel viability assay reveals differences in fungicide sensitivity of root endophytic fungi originating from fungicide-exposed and non-exposed habitats

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Dark septate endophytes (DSEs) are taxonomically diverse fungi that ubiquitously colonize plant roots, yet their responses to fungicide exposure remain unexplored. We investigated fungicide sensitivity in Periconia sp. isolates originating from two distinct habitats: natural sandy grasslands in Fülöpháza and intensively managed agricultural fields in Martonvásár, to determine whether fungicide sensitivity reflects the habitat. Thirty isolates (15 per site) were identified through sequencing and phylogenetic analysis of ITS (internal transcribed spacer), LSU (partial 28S large subunit rDNA), SSU (partial 18S small subunit rDNA), TEF1-α (translation elongation factor 1-alpha), and RPB2 (RNA polymerase II second largest subunit), and their sensitivity to the demethylation inhibitor (DMI) fungicide difenoconazole was assessed. We developed a standardized inoculum preparation for filamentous fungi. We adapted a high-throughput microtiter plate viability assay, applying a resazurin-based fluorescent dye to quantify fungal metabolic activity across concentration gradients of difenoconazole. This approach offers a novel and scalable method for assessing fungicide sensitivity in filamentous, non-sporulating fungi. Our results revealed significant habitat-dependent differences: isolates originating from agricultural habitats displayed a stronger growth under difenoconazole treatment compared to those from grassland populations. These findings provide experimental proof that the use of fungicides in agricultural settings can influence fungicide sensitivity in fungal endophytes. By integrating this new methodological approach with ecological considerations, our study presents new opportunities to understand better how fungicide use affects plant-associated fungal communities beyond just plant pathogens. Keywords: dark septate endophytes, fungicide resistance, habitat adaptation, fungal growth assay

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IMPORTANT VIRUS AND VIRUS-LIKE DISEASES IN SLOVENIAN VINEYARDS AND POSSIBILITIES FOR THEIR MANAGEMENT

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In recent years, the most important virus and virus-like diseases affecting Slovenian vineyards are caused by grapevine fanleaf virus (GFLV) and Grapevine Flavescence dorée phytoplasma. The vectors of both pathogens are present in Slovenia; Scaphoideus titanus is widespread, while the nematode Xiphinema index has so far been found only in Primorska region in south-western part of Slovenia. GFLV related issues escalated in the last 10 years, leading to high yield reductions in many vineyards in the Primorska region, with losses exceeding 90% in some cases. Effective management options of nematodes in such vineyards are very limited. Although crop rotation and fallow periods can reduce nematode populations, these strategies are not economically feasible due to the extended period required before vineyard replanting. In frame of the national project Resense-Vitis we are testing alternative management strategies to reduce the nematode populations, shorten the period during which the virus can be transmitted and consequently shorten the excessively long replanting period. To accomplish these objectives, we have selected the experimental vineyards, prepared the disease evaluation protocols and have implemented regular disease monitoring. We have optimized GFLV detection in nematode vectors and set up both field and pot trials with bio-fumigation and alternative cover crops. At the end of these experiments we will assess the reduction in nematode populations and their ability to transmit the virus by detecting the presence of GFLV in the nematodes.
The work was funded by the Ministry of Agriculture, Forestry and Food of Slovenia, and Slovenian Research and Innovation Agency (ARIS Project L7-50153).

Revealing the function of Hydrophobic surface binding proteins in the pathogenicity of *Mucor lusitanicus*

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Hydrophobic surface binding proteins (HsbA) are antigenic galactomannoproteins covalently anchored to the fungal cell wall. They play a crucial role in adhesion and surface penetration by recruiting hydrolytic enzymes such as cutinase in plant pathogenic fungi. The immunogenic Mp1 protein of *Talaromyces marneffei* harbors a conserved HsbA domain that modulates host inflammatory responses via arachidonic acid binding. In this study, we investigated *hsbA* knockout (*ΔhsbA1-5*) and overexpression mutants (pAV1–3) of *Mucor lusitanicus*, a model organism of mucormycosis. Quantitative PCR revealed coordinated transcriptional activity among *hsbA* genes. Growth assays showed significantly altered profiles of knockout and overexpression mutants at different temperatures. Expression changes, either by knockout or overexpression, generally increased resistance to cell wall stressors, except in pAV1. Under SDS-induced membrane stress, *ΔhsbA2* and pAV3 mutants displayed modest but statistically significant growth reduction. The pAV3 mutant showed enhanced sporulation but reduced metabolic activity in XTT assays. All overexpression mutants exhibited a significant reduction in germination capacity. During biofilm formation, a protective extracellular matrix (ECM) shields microorganisms from host immunity, often promoting growth and chronic infection. Scanning electron microscopy revealed net-like ECM structures in overexpression mutants, while the control strain produced granule-like ECM. Cell

surface hydrophobicity, a virulence factor influencing adherence to diverse surfaces (plastic, glass, metal, epithelial cells), was quantified by MATH assay. Interestingly, hydrophobicity was significantly increased in several *hsbA* mutants. Notably, HsbA overexpression reduced virulence in *Drosophila melanogaster* and *Galleria mellonella*, whereas knockout mutants exhibited increased virulence. Our findings underscore the importance of the HsbA protein family in regulating fungal growth, surface properties, stress responses, biofilm formation, and pathogenicity in *M. lusitanicus*, positioning it as a promising molecular target for antifungal strategies and mucormycosis intervention.
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Drought shapes fungal communities of grapevine leaves in the Eger wine region

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As a result of ongoing climate change and unsustainable land management practices, drought has become a major challenge affecting plant health and productivity in both natural and agricultural ecosystems. Shifts in environmental conditions impacting plants are likewise to alter the composition and function of their associated microbial communities. While the adaptive responses of the plant are vital for drought tolerance, increasing evidence suggests that plant-associated microbial communities also play an essential role in shaping plant resilience to water deficit. Over a three-year period, we investigated the impact of drought stress on grapevine-associated fungal communities in the Eger wine region. Sampling sites were delineated from prior empirical observations, with spatial heterogeneity in water availability assessed via drone-based remote sensing and in situ physiological measurements. We collected healthy-looking leaves from three different cultivars (Furmint, Kadarka, Syrah) in the summer of 2020, 2022 and 2023. Using DNA metabarcoding, we compared fungal richness, abundance, and community composition in leaf samples from control plots and those subjected to drought stress. Drought affected the richness, abundance and composition of certain fungal groups, such as wood saprotrophs, in almost every cultivar and sampling year, while its impact on other groups was observed only in specific cultivars or years. Across sampling years, we also consistently observed changes in richness, abundance, and community composition. Our findings show that drought stress can substantially alter grapevine-associated fungal communities, with effects varying among functional groups, cultivars, and years. Such insights can contribute to predicting vineyard ecosystem responses to climate change and may support adaptive management strategies aimed at sustaining grapevine health and productivity in increasingly drought-prone regions.
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Biocolors - Synthetic biology to produce *Cortinarius* anthraquinone pigments

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Anthraquinones in the emodin family are produced by bacteria, fungi, and plants. They display various biological activities exploited e.g., for crop protection, and may also be utilized as sustainable, bio-based colorants for the textile, paints, electronics, and cosmetic industries. Anthraquinone pigments from Cortinarius mushrooms have been used for artisan dyeing because they are stable, colorfast, and compatible with various dyeing methods. However, their chemical synthesis is complex and uneconomical, and harvesting wild mushrooms from forests in commercial quantities is not feasible. Here, we use genomics, transcriptomics, and synthetic biology to uncover the biosynthesis of the anthraquinone scaffold compounds emodin and endocrocin, and their methylation to the yellow pigments physcion and dermolutein in Cortinarius semisanguineus and C. sp. KIS-3. Both the nonreducing polyketide synthases (nrPKSs), and the regiospecific, fastidious O-methyltransferases (OMTs) are non-orthologous to their Ascomycete counterparts, suggesting a parallel evolutionary origin for the pathway in Basidiomycetes. The genes for the nrPKS and the OMTs are not all clustered in Cortinarius, revealing metabolic crosstalk among paralogous nrPKS biosynthetic gene clusters.
Heterologous biosynthesis of physcion and dermolutein in Saccharomyces cerevisiae opens the way to produce specific Cortinarius anthraquinones, and to modify these scaffolds to tune their chemistry towards their various applications.

The possible mechanism of the Esca disease-promoting effect of *Aureobasidium pullulans*

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Esca syndrome belongs to the group of trunk diseases, a group of vascular fungal infections of the grapevine. The pathogenesis of Esca is still unknown and believed to be affected by several factors like host stress, climatic conditions, as well as the interactions between the members of the grapevine microbiome. Our previous pathogenicity tests on grapevine cuttings revealed that the dimorphic fungus Aureobasidium pullulans (Apu) may positively affects Esca disease development, which phenomenon is achieved by the interaction of Apu with the Esca pathogenic fungus Phaeomoniella chlamydospora (Pch). The results of the present study suggest that this effect is based on the cumulative or synergistic interaction between Apu exopolysaccharides (EPS) and Pch secreted proteins (SP). Grapevine cuttings co-infected with a Pch and Apu strains showed severely necrotized leaves after two weeks. Water extracts of these leaves were prepared and used for phytotoxicity assays against detached grapevine leaves, resulting in a dose-dependent toxic effect. This suggests the severe symptoms of the co-infected plants are the result of phytotoxin accumulation. Exopolysaccharide fraction of Apu was prepared by ethanol precipitation, while secreted protein fraction of Pch was prepared by trichloroacetic acid precipitation from liquid cultures. The toxicity of Apu EPS, Pch SP, and their mixture was tested on onion cell suspension. Apu EPS did not show any negative effect, but its presence significantly increased the phytotoxicity of Pch SP. Apu EPS was visualized by microscopy and congo red staining. The EPS seems to attach to the surface of plant cells.
Based on the results mentioned above, we hypothesize that the EPS of Apu and the SP of Pch may interact with each other, resulting in the development of severe symptoms on the leaves of infected grapevines. We think that Apu EPS may act as an “anchor” between the host cell surface and Pch phytotoxic proteins.

First confirmed occurrence of *Uromyces muscari* on grape hyacinth in Hungary

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Muscari species are widely cultivated in Europe as ornamental plants due to their aesthetic value. However, they are vulnerable to infections by rust fungi, which can diminish both their visual quality and overall vitality. Although Uromyces muscari has previously been found on several Muscari species in Hungary, its occurrence on Muscari armeniacum had not been confirmeduntil now. In May 2023, rust-like symptoms were observed on the leaves and flower stalks of M. armeniacum plants in a private garden located in Solymár, Hungary. The causal agent, designated as isolate UmHu2, was identified through both morphological examination and molecular phylogenetic methods. DNA was extracted, and specific genomic regions—namely, the internal transcribed spacer (ITS), the large subunit (LSU) of nuclear ribosomal DNA (nrDNA), as well as β-tubulin1 and translation elongation factor 1-alpha (TEF1)—were amplified and sequenced. Comparison of these sequences with entries in the NCBI GenBank database revealed 98–100% identity with the corresponding sequences of the Canadian reference strain U. muscari DAOM 75626 (ITS-LSU: PQ637652; β-tubulin: PQ558638; TEF1: PQ619463). Phylogenetic analysis based on these four loci clustered the Hungarian isolate UmHu2 with other known U. muscari strains from Hungary (Um28028BP, Um37343BP, Um97768BP), California (3465) and Canada (DAOM 75626), thereby confirming its identity as Uromyces muscari (Duby) Lév. To assess pathogenicity, healthy, five weeks old M. armeniacum plants , were artificially inoculated with teliospores of the UmHu2 isolate. The fungus was subsequently re-isolated and examined microscopically, and DNA sequencing confirmed that it matched the original inoculum. This fulfilled Koch's postulates, proving the causal relationship between the fungus and the observed symptoms. We conducted cross-inoculation experiments with Uromyces muscari isolates UmHu1 and UmHu2, derived from Muscari neglectum and M. armeniacum, respectively. Both isolates successfully infected and caused symptoms on both Muscari species, irrespective of their host of origin. However, no symptoms developed on Hyacinthus orientalis, suggesting that U. muscari is specific to the genus but not restricted to a single species. To the best of our knowledge, this is the first confirmed report of U. muscari infecting M. armeniacum in Hungary.

bZIP-type transcription factors in *Aspergillus flavus* and *Fusarium verticillioides*

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Aspergillus flavus is a saprotrophic and pathogenic fungus, which causes severe agricultural damage to crops, often lethal aspergillosis in humans, and triggers liver damage and immunosuppression in mammals through the production of the secondary metabolite aflatoxin. Fusarium verticillioides is the phytopathogen of cereals and it is also capable of producing mycotoxins like fumonisin. In filamentous fungi bZIP type transcription factors are crucial elements in governing stress response and production of secondary metabolites like mycotoxins. In this work we examined bZIP transcription factors in A. flavus and F. verticillioides. For this purpose, we generated gene deletion and complementation mutants, verified by PCR tests. Oxidative, osmotic, cell wall integrity and heavy metal stress sensitivity studies were also performed on the wild type as well as the mutant strains. We also determined the mycotoxin production of the strains. Based on our results, the deletion mutants showed sensitivity to oxidative stress, in some mutants we detected alteration in the mycotoxin production, while the complemented and the wild type strains showed similar phenotype. We also started corn seed infection experiments to determine the virulence of the mutants. To

decipher the gene regulating patterns of some A. flavus bZIP-type transcription factors by ChIP-seq analysis, we created 3X FLAG-tagged complemented strains. In the following we will also carry on RNA sequencing to identify the genes regulated by the studied transcription factors. We plan to start Galleria mellonella infection assays with A. flavus strains to determine the virulence of the mutants compared to the wild type. Hopefully, our basic research can be exploited for agriculture, drug development and food industry.

Current trends in human West Nile and Usutu virus infections in Hungary

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Background: West Nile virus (WNV) and Usutu virus (USUV) are mosquito-borne orthoflaviviruses endemic to Hungary and much of Europe. The first West Nile neuroinvasive disease (WNND) cases in Hungary were documented in 2004, while the first USUV-related meningitis case was diagnosed in 2018. Materials and Methods: In Hungary, laboratory diagnosis of human arbovirus infections is exclusively performed at the National Reference Laboratory for Viral Zoonoses of the National Center for Public Health and Pharmacy (Budapest). The serological diagnosis of acute infections is based on parallel testing of serum and cerebrospinal fluid (CSF) samples for all endemic orthoflaviviruses using indirect immunofluorescence and ELISA methods. Anticoagulated whole blood and urine samples collected during the early phase of illness are essential for molecular diagnostic assays. Due to the close genetic and serological relatedness between WNV and USUV, RT-qPCR testing for both viruses must always be performed simultaneously. Results: During the 2024 transmission season, a total of n=113 WNV cases were reported in Hungary, of which 92% were WNND and 8% were classified as West Nile fever. Three human neuroinvasive USUV infections were confirmed, including one with a fatal outcome. At least 20% of confirmed WNV cases required treatment in intensive care units. An elevated proportion (11%) of immunocompromised patients was also observed, with most (n=8) having hematological malignancies. WNV infections were reported from nearly all parts of the country; however, Fejér County in the Central Transdanubian region had a notably high incidence rate (IR=5.72). A seasonal shift was also observed in 2024, with the first WNV infections detected in mid-July (one month earlier than in previous years), suggesting a prolonged transmission season. Conclusions: The number of WNV cases reported in 2024 significantly exceeded the annual case numbers of recent years, although it remained lower than the peak recorded in 2018. The geographical spread of WNV in 2024 was the most extensive ever recorded in Europe, covering n=204 regions across nineteen countries. Although at the time of submission (June 2025), the 2025 WNV and USUV transmission season has not yet begun, we believe that comparative analysis of epidemiological and microbiological data from subsequent seasons will be a valuable addition to current knowledge about these emerging pathogens.

SIGNIFICANCE OF HIV-1 CAPSID IN INITIATION OF INFECTION

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HIV/AIDS is still a serious health issue worldwide. The detailed analysis of the molecular events of viral replication has resulted new intervention possibilities in the treatment of AIDS. HIV-1 capsid performs multiple functions, one of which may be to enhance the efficiency of reverse transcriptions. We provided research evidence that HIV-1 protease functioning not only at the late phase of virus replication to cleave proteins to final size, but at the early phase of viral replication cycle contributing to loosing nucleocapsid protein structure in order to enhance viral reverse transcription. The involvement of retroviral PR in the early stages of viral replication cycle was suggested on the basis of in vitro experiments with purified capsids. Our studies demonstrated a novel retroviral protein processing pathway mediated by the viral PR that involves the regulated in situ cleavage of mature nucleocapsid protein assembled with the viral RNA. Such a proteolytic event is required for the progression of virus replication in the early phase of infection. The results suggested, that in the presence of PR inhibitors not the initiation and progression of reverse transcription is blocked, but the stability of full-size unintegrated cDNA, which is affected in the presence of PR inhibitor. Research defined the role of retroviral proteases in the generation of mature Gag-related protein products, which comprise the virus particle structure. This work played a central role in the development of HIV protease inhibitors, which became a mainstay in the treatment of HIV-infected individuals. Potent proteinase inhibitors able to halt these processes resulting inhibition of HIV-1 replication and integration. Nucleocapsid protein likely also to facilitates replication. Based on newest analysis on the role of viral capsid revealed that capsid mutations and small molecular inhibitors that alter viral capsid stability can also inhibit viral reverse transcription in infected cells. With the discovery of lenacapavir (Sundquist et al) which was chosen the breakthrough of the year 2024 by the Science Magazine a new class of AIDS drugs - the capsid inhibitors - were provided for the effective AIDS therapy. These can be administered as an injection, only twice a year, resulting almost one hundred percent of preventing HIV infection in thousands of patients participated in the trial.

Antibiotic resistance genes screening in staphylococci selected from raw goat milk

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Raw goat milk represents functional food because of its nutritionally available components, beneficial microbiota including. Besides beneficial microbiota, contaminant bacteria can occurr in raw goat milk. Mostly non-requested bacteria are those resistant to antibiotics, especially containing antibiotic resistance genes. Among contaminant microbiota belong also staphylococci. Therefore, the aim of this study was to check presence of different antibiotic genes in staphylococcal strains isolated from raw goat milk to map condition and also to have in mind use of postbiotics (bacteriocins) to prevent and/or eliminate them. Twenty seven (27) different species strains were tested to have or not to contain 16 genes responsible for antibiotic reistance (mecA, mecC, blaZ, ermA, ermB, ermC, msrA, tetK, tetL, tetM, catpC221, catpC194, catpC223, aac(6)-Ie-aph(2)-Ia, vanA, vanB). The appropriate primers and protocols were used as previously published. Visualization was provided using GelDoc Go Imaging System (Bio-Rad Laboratories, USA). Among tested staphylococci were

involved the species Staphylococcus hominis, S. warneri, S. epidermidis, S. arlettae, S. equorum, S. lentus, S. xylosus, S. capitis, S. sciuri, and S. delphini identified using MALDI-TOF mass spectropmetry. Nine (9) strains contained MecC gene encoding oxacillin, penicillin and methicillin resistance. However, mecA gene with the same coding function was not detected. BlaZ gene coding resistance to β-lactams was detected in 2 species strains (S. xylosus and S. hominis). Staphylococci did not posses ermA, ermB, and ermC genes coding erythromycin resistance similarly as tetK, tetL, and tetM genes coding tetracykline resistance. Only chloramphenicol gene cat (pC194) was present in 3 strains. The other genes were not found. To know antibiotic genes profile in contaminant staphylococci in raw goat milk allows us to decide for a suitable eliminating/preventig strategy. Promising approach is use of postbiotics (bacteriocins).

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Effect of Enterocin 7420 on intestinal microbiota and immunity of mice infected with methicillin-resistant *Kocuria varians* R33/4

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The increasing number of methicillin-resistant (MR) staphylococci, mosly MR S. aureus (MRSA) poses serious threats to modern healthcare, veterinary medicine and food safety. Besides MRSA, MR coagulase-negative staphylococci and related species – Kocuria spp., may be considered as significant sources of antibiotic resistance. Reducing the MRS prevalence in animals (pets, livestock) represents a major challenge, as these resistant strains have negative impact on animal health and productivity as well as the consumer´s safety. Bacteriocins/enterocins (Ent; antimicrobial proteinacous substances produced by beneficial strains of lactic acid bacteria/enterococci) as a biggest group of postbiotics have attracted attention as potential antimicrobial compounds to reduce or eliminate MR bacteria and prevent their infections. The aim of this study was to evaluate the impact of the postbiotic substance Ent7420 on the intestinal microbiota and immune response (secretory IgA) of BALB/c mice experimentally infected with MR K. varians R33/4 strain. Forty mice were divided into four groups: S (MR K. varians R33/4; 1.0x105 CFU/ml; 100 µl/animal/day p.o. between 0-7 days to simulate a pathological attack), E (Ent7420; 50 µl/animal/day p.o., between 8-21 days), S+E (combined application of MR strain between 0-7 days and Ent7420 between 8-21 days) and C (control; without additives). Mice were monitored between days 21-42 (after substances withdrawal). The MR strain was able to colonized the digestive tract of rabbits, reaching counts in the range 1.55–2.73 log cycle. Mice receiving the MR R33/4 (groups S) showed a temporary decrease in weight (-0.42 g) compared to C data. Increased sIgA levels suggesting the pathological attack of MR R33/4. The antibacterial effect of Ent7420 was noted due to reduction of MR R33/4 strain, MRS (by 0.2 log cycle), coliforms ($p<0.01$) in E and E+S group compared to groups S and C. These results showed that MR K. varians R33/4 exerted a negative impact on gut health, whereas Ent7420 exhibited beneficial modulatory effects on gut microbiota and immunity. These findings support the potential of Ents as postbiotic tools in managing MR bacterial colonization. Tested Ent7420 could be used as feed additive in animal nutrition with medicinal effect to stabilize and improve the animals health during a possible bacterial infection.

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Multipathogenic wastewater surveillance: streamlining for multiple targets from a single sample

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Wastewater acts as a community health fingerprint, reflecting spatio-temporal population health changes. Wastewater-based epidemiology (WBE) has been used for decades, but it gained increased interest during COVID-19 pandemic. Evolving from early poliovirus monitoring, surveillance is now optimized for diverse targets worldwide. In Hungary, we developed a standardized pipeline to analyse samples from 27 sampling locations (including wastewater treatment plants (WWTPs) from every county seat, three major WWTPs from Budapest, WWTPs from five of the agglomeration cities, and the International Airport), covering ~3.9 million people with weekly frequency. Sample processing includes centrifugation, with 50 ml supernatant concentrated via an in-house flat sheet membrane (>270 kDA cut-off) ultrafiltration technique. Poliovirus detection uses a different branch: processing higher sample volume (1000 mL) and commercially available filter membranes (Amicon 10, >10 kDA cut-off) for higher sensitivity. Nucleic acid is extracted from concentrates (QIAamp Viral RNA Mini Kit) and pellets (Qiagen Power Soil Pro Kit). Downstream analysis relies on d(RT-)PCR, (RT-)qPCR, and NGS methods. The method is used to detect several parameters: respiratory viruses (SARS-CoV-2, Influenza, RSV), viral, bacterial, and protozoan enteric pathogens (norovirus, rotavirus, Salmonella, Campylobacter, Cryptosporidium, C. difficile), and 7 AMR genes. All parameters, except poliovirus, are incorporated to the central processing branch. This multi-target surveillance system enables simultaneous pathogen detection via a simple, flexible pipeline. It can incorporate new targets without significantly increasing workload or costs, with minimal sensitivity loss. The pipeline supports flexible, work- and cost-efficient multi-target surveillance with good molecular detection recovery and consistency. However, the inclusion targets with significantly different characteristics (e.g. very small size or easily degradable genome) has proven to be challenging. This research was supported by EU-WISH (No. 101140460).

Three hungarian hazelnut clones show reduced powdery mildew severity from *Erysiphe corylacearum*

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The demand for common hazel (Corylus avellana) fruit is steadily increasing. However, cultivation has been severely hindered by an invasive fungus of Asian origin, Erysiphe corylacearum, which has spread across Europe over the past decade. Its economic importance lies in its ability to infect hazelnut fruits, significantly reducing both yield and quality. To support management strategies, our aims were to (i) monitor symptom development throughout the growing season and (ii) evaluate differences in disease severity among hazelnut clones and varieties. Hungarian clones and foreign varieties, ten altogether, were assessed over three consecutive years (2022–2024) at the orchards of the Research Centre for Fruit Growing, Érd, Hungary. The timing and progression of symptoms were recorded. Three times per year (early summer, late summer, and mid-autumn), 100 leaves per clone or variety were collected and rated for disease severity on a six-level scale. An aggregated disease index was calculated, and analysis of variance (ANOVA) was conducted to test for differences in

disease severity among varieties and clones. Games–Howell post hoc tests were then applied for pairwise comparisons. Symptoms first appeared from mid- to late May, depending on the variety and year. By early June, colonies up to 2 cm in diameter developed on leaves, followed by gradual expansion and the appearance of fruiting bodies from late June to mid-August, which became gregarious by late August or early September. Significant deformation of young, fruit-bearing shoots was also noted. Statistical analysis revealed significant differences in disease severity, with 'Fehér Lambert', 'Cosford K.2', and 'Bőtermő Nagy', three clones of Hungarian origin exhibiting significantly lower disease indices. These findings support variety selection decisions and highlight the potential role of Hungarian clones in managing E. corylacearum.
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Quorum sensing and biofilm formation: Dual drivers of virulence in *Phaeomoniella chlamydospora*

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Esca is one of the significant grapevine trunk diseases. Ascomycete fungus Phaeomoniella chlamydospora (Pch) is notably important and is known as a pioneering pathogen in the early stages of disease development. Our previous research demonstrated that Pch isolates are capable of forming biofilms in vitro, accompanied by the production of an extracellular matrix and morphological dimorphism of vegetative cells and these processes likely positively regulated by acetic acid functioning as an endogenous signaling molecule. We hypothesize that the acetic acid-mediated quorum sensing process may also play a crucial role in regulating other virulence factors. In this study, three Pch isolates (P46, P201, and P621) were investigated to assess the influence of acetate on the expression of selected virulence-associated traits. The fungal strains were cultured on solid media supplemented with increasing concentrations of acetic acid (6.25 mM-100 mM, in a five-point, two-fold dilution series). Our observations revealed that low acetate concentrations promoted pigment production, whereas higher concentrations inhibited this process. The ability of the fungus to degrade plant material also examined. Amylase, cellulase and protease activities were significantly enhanced under low acetate conditions, while high concentrations suppressed their expression. In contrast, esterase activity remained comparable to the control at low acetate levels but was reduced at higher concentrations. Pectinase and phenol-degrading activities showed a consistent decrease across all acetate concentrations tested. All three Pch strains examined in liquid media, displayed an acetate dependent reduction in antibacterial properties toward four Pseudomonas strains, isolated from grapevine xylem tissues. Based on our earlier findings, we propose that the acetic acid-mediated quorum sensing mechanism significantly influences the regulation of virulence factors in Pch. This mechanism exhibits a complex, density-dependent behavior: initially, at low cell densities, it promotes specific key events linked to infection, whereas at higher cell concentrations, it inhibits these same processes. Conversely, during the advanced stages of infection, quorum sensing consistently facilitates the persistence of the pathogen.
This research was supported by the TKP2021-NKTA-16 research project.

Application of Raman Spectroscopy for Phenotypic Differentiation of Carbapenem-Resistant *Klebsiella pneumoniae*

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The emergence and spread of carbapenem-resistant Klebsiella pneumoniae (CRKP) strains represent a major therapeutic challenge. The timely initiation of targeted antimicrobial therapy is often delayed due to the time requirements of conventional resistance detection methods, including PCR and phenotypic assays. Our study aimed to investigate whether Raman spectroscopy can differentiate carbapenem-resistant phenotypes from other resistance types in K. pneumoniae. We analyzed isolates with various resistance profiles, including CRKP, ESBL-producing, naturally resistant, and strains resistant to aminopenicillins or trimethoprim-sulfamethoxazole. After overnight culture, bacteria were suspended in distilled water and mixed with an equal volume of negatively charged silver nanoparticles (AgNPs), synthesized from silver nitrate and sodium citrate following published protocols. The mixture was applied onto silicon slides and air-dried. Raman spectra were recorded using a Thermo Fisher DXR Raman microscope (780 nm, 12 mW, 50 µm slit, 50× magnification, 16 accumulations of 2 s). Spectral range: 3300–200 cm^{-1}. Preprocessing included cosmic ray and fluorescence correction. Spectra were averaged and evaluated by group. The CRKP group was consistently distinguishable from other phenotypes. The most pronounced differences were observed in the 600–750, 950–1050, 1200–1300, and 1550–1650 cm^{-1} regions, corresponding to cell wall, protein, and nucleic acid vibrational modes. In these ranges, CRKP strains showed significantly higher intensities and distinct peak patterns compared to ESBL or intrinsically resistant strains. K. aerogenes, used as a species control, displayed clearly separate spectra, supporting the method's species specificity. Raman spectroscopy sensitively reflects cellular changes associated with carbapenem resistance. Clinically, this may support empirical therapy planning and infection control by providing rapid phenotypic discrimination. With an appropriate database and analytical framework, Raman spectroscopy has the potential to become an effective diagnostic tool for early CRKP identification.

Genomic characterization of a multidrug-resistant O23:H16-ST453 *Escherichia coli* strain cultured from broiler chicken faeces in Hungary

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A multidrug-resistant (MDR) E. coli isolate designated strain K1G was cultured from the rectum content of a Ross-308 rooster on violet red bile glucose (VRBG) agar plate containing 16 mg/l gentamicin. The host animal was fed regular control feed without growth promoting antibiotics. The rooster received the coccidiostat Monensin Na (at 110 mg/kg) in the starter and grower diets. E. coli strain K1G is a serotype O23:H16 and sequence type ST453 isolate displaying resistance to ampicillin, cefotaxime, ceftazidime, ciprofloxacin, and gentamicin according to current EUCAST MIC breakpoints, however, resistance for many additional antibiotics (streptomycin, spectinomycin, sulfamethoxazole, chloramphenicol, florfenicol, tetracycline, nalidixic acid) was also detected. Whole genome sequencing (WGS) on Illumina MiSeq and Oxford Nanopore platforms was performed to map resistance determinants, virulence factors, and mobile elements (using CARD, CGE ResFinder/VirulenceFinder; ISFinder). The E. coli K1G chromosome lacked acquired antibiotic

resistance genes (ARGs) but encoded a number of mutation-associated resistance loci, virulence factors, and insertion sequences. The strain harboured three plasmids: pEc_K1G_A (113 kb, phage-like, low GC, no ARG/virulence); pEc_K1G_B (86 kb mosaic, dual replication, blaTEM-1b, mer module, aerobactin, efflux, colicin V/microcin traits, ~31% mobile DNA); and pEc_K1G_C (IncC type 1, three ARG islands, mer modules, extensive IS26-mediated rearrangements, no virulence). Mating with E. coli TG1Rif showed no conjugative transfer of resistance determinants, however, pEc_K1G_C could be mobilized with a low efficiency by the aid of helper plasmids and complementation by SGI1. MIC assays probed responses to puromycin, Hg2+, Co2+, and TeO3(2-). Observed phenotypes did not include predicted puromycin or mercury resistance under the test conditions applied, suggesting context dependent gene expression/functions. Our findings highlight complex genomic plasticity enabling horizontal exchange of resistance traits in poultry microbiomes and underscore integrative nutrition-immunity-microbiota strategies for sustainable gut health. Moreover, the carriage of multiple acquired ARGs in E. coli K1G shows that individual chickens can harbour or develop antibiotic resistance in the absence of direct antibiotic exposure.

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Potential of Enrichment cultures in the bioremediation of groundwater contaminated with MTBE

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Abstract: Methyl tert-butyl ether (MTBE) and BTEX compounds (benzene, toluene, ethylbenzene, and xylenes) are prevalent groundwater contaminants primarily resulting from petroleum-related activities. MTBE, widely used as a fuel oxygenate to improve gasoline combustion and reduce air pollutants, is of particular concern due to its high mobility in the subsurface and resistance to conventional remediation techniques. BTEX compounds, which are frequently co-detected with MTBE, pose a significant environmental concern due to their toxicity and persistence. Among the various remediation approaches explored, bioremediation, utilizing native microbial communities capable of degrading these compounds, has emerged as a sustainable and effective alternative. Given the common co-occurrence of MTBE and BTEX, stimulating indigenous microbial consortia in situ may offer a synergistic strategy for the concurrent degradation of both contaminant groups. This study investigated the microbial community structure at a site heavily impacted by MTBE and BTEX pollution to identify potential microorganisms capable of degrading MTBE. Enrichment culture techniques were employed to promote the growth of MTBE-degrading populations, and solid-phase isolation methods were used to recover and characterize individual degraders from both field samples and enrichment cultures. The findings contribute to the development of bioremediation strategies tailored to complex contaminant mixtures in groundwater systems.

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Investigation of structural stability of B1N680, a *Solanum lycopersicum L.*-derived defensin

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The spread of fungicide-resistant phytopathogenic fungi is a serious problem in agriculture. Due to the decreasing effectiveness and environmental concerns of currently used chemical-based pesticides, new environmentally friendly antifungal strategies have become the focus of research. The defensins of plant origin are promising biofungicide candidate, because of their board spectrum of antifungal activity, lack of cytotoxicity effect and high environmental stability. In our previous work, we identified and heterologously produced a Solanum lycopersicum L.-derived defensin (B1N680). We have determined that the recombinant protein inhibits the growth of several filamentous fungi (Botrytis cinerea, Cladosporium herbarum, Fusarium species) at low minimal inhibitory concentration (6.25-25 µg/ml). However, for its broad agricultural applicability, it is important to examine the B1N680 antifungal effects under different environmental conditions. Therefor we aimed the investigation of pH-, thermo-, UV- and salt tolerance of the defensin in susceptibility test against C. herbarum. According to our results, B1N680 preserved its antifungal activity against C. herbarum under slightly acid (pH=6) and basic (pH=8) conditions, as well as after heat-treatment (50°C, and 100°C) and UV exposure (30 min). The electronic circular dichroism (ECD) spectroscopy measurements demonstrated that B1N680 has remarkable structural stability as maintaining its intact structure even at 95°C and after UV-radiation. However in the present of salts (100 mM NaCl and 2 mM CaCl2) the defensin lost its antifungal activity. The abovementioned results demonstrate that the B1N680 preserves its antifungal effect under extreme conditions (pH=6-8, 100°C, UV-treatment), therefor after field experiments, it can be used as a potential biofungicide in the future.

Tracking Aphid-Transmitted Viruses in Invasive Weeds: Plant Hosts or Insect Contamination?

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In this study, we investigated the virome of invasive weed species: Erigeron canadensis, Erigeron annus, and Solidago canadensis, belonging to the family Asteraceae. Samples were collected in 2017 from agricultural areas near Tusnád and Segesvár, Transylvania. The selected species were not only grown as invasive weeds but also served as hosts for aphid species. Using high-throughput sequencing of RNA and small RNA, we characterised the virome of these weeds and detected the presence of Wuhan Aphid Virus 2 (WHAV2) and Wuhan Insect Virus 21 (WhIV21). WHAV2 belongs to the Jingmenvirus group of segmented flavi-like viruses. It was first described in 2013 from aphid species in Wuhan, China (Shi et al., 2016b). In 2020, it was detected in Pisum sativum samples in France, marking both the first report of the virus in a plant host and its first detection in Europe (Gaafar et al, 2020b). WhIV21 was first identified in 2016 from insect samples during a large-scale metagenomic survey of invertebrate viromes (Shi et al., 2016a), detected later in various arthropod hosts as well as in P. sativum (Gaafar et al., 2020a), but its biology, host specificity, and transmission remain poorly understood. In this study, WHAV2 was detected in all three plant species at both locations, while WhIV21 was found only in E. canadensis and E. annuus at Tusnad. In order to clarify the true origin of these viruses by determining whether their detection reflects genuine infection of

plant tissue or the presence of infected aphid vectors, we tested the presence of the insects using COI gene-specific RT-PCR and detected B. helichrysi in several samples. While WhIV21 was found exclusively together with the aphid, WHAV2 was detected in samples where no insect material could be identified, suggesting true infection of the plants with this virus. Our findings suggest that invasive weeds may play a role in shaping virus ecology, serving as reservoirs for various plant- and insect-infecting viruses. Future work should assess these viruses' roles and refine weed management in integrated pest control.
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Comparative analyses of phenotypic and genotypic antibiotic susceptibility of *Pasteurella multocida*

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Pasteurella multocida is responsible for a wide variety of animal diseases worldwide, causing major economic losses. These infections are usually treated with antibiotics, however, the emergence of multidrug-resistant (MDR) strains is increasingly hindering. Understanding antibiotic resistance in P. multocida is important for effective treatment strategies and public health, as it impacts both animal and human welfare. The aim of this study was to evaluate the antibiotic susceptibility of 80 P. multocida isolates using phenotypic (disc diffusion and broth microdilution) and genotypic analysis via whole genome sequencing, with focus on the occurrence of MDR strains. The strains were tested with nine antimicrobial classes (penicillins, cephalosporins, aminoglycosides, tetracyclines, macrolides, fluoroquinolones, lincosamides, phenicols, sulfonamides). Antimicrobial resistance gene (ARG) sequences, single-nucleotide polymorphisms (SNPs) and the prevalence rate of ICE-Pmu were evaluated in paired reads using the Bacterial and Viral Bioinformatics Resource Center (BV-BRC, https://www.bv-brc.org/) and the Comprehensive Antibiotic Resistance Database (CARD) by Resistance Gene Identifier (RGI) (https://card.mcmaster.ca/analyze/rgi), respectively. Phenotypic results indicated that cephalosporins and phenicols are the most effective drugs for the treatment of pasteurellosis, but the majority of strains had high sensitivity to fluoroquinolones and tetracyclines as well. In contrast, high resistance rates were observed with sulfamethoxazole and clindamycin. The phenotypic results for phenicols, tetracyclines and fluoroquinolones showed a strong correlation with the detected resistance genes, however, resistance to sulfamethoxazole, β-lactams, and macrolides remains genetically unexplained, suggesting the existence of additional resistance mechanisms that remain to be explored.

New and recently described root-colonizing endophytic fungal taxa from gramineous plants in semiarid grasslands and agricultural fields

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Fungal root endophytes are widespread symbiotic partners of various plant species, and colonize the cortical cells and intercellular regions of roots. These fungi cause no symptoms or tissue damage to their hosts, at least during some period of their life cycle. Many of these fungi, most of which belong

to the phylum Ascomycota, have pigmented hyphae and are commonly referred to as “dark septate endophytes” (DSE). These fungi are widely distributed in several biomes and climatic regions, including arid, semiarid, and temperate grasslands. During our investigations of root-colonizing fungi of gramineous plant species of agricultural fields and semiarid grasslands, we found that several isolates represent novel lineages. Thus, we aimed to identify these isolates and clarify the phylogenetic position of the new lineages. The roots of Festuca vaginata and Stipa borysthenica were sampled in the semiarid grassland of the Great Hungarian Plain, and the roots of Triticum aestivum were sampled in croplands. Genomic DNA was extracted and three loci (ITS, partial 28S and 18S regions of nrDNA) were amplified and sequenced from 33 isolates. Based on these sequences the isolates grouped into three orders: Pleosporales, Magnaporthales and Sordariales. Additionally, the translation elongation factor 1-alpha gene (TEF1-α) and the RNA polymerase II second largest subunit gene (RPB2) were amplified and sequenced from isolates belonging to Pleosporales; the RNA polymerase II largest subunit gene and TEF1-α from Magnaporthales; and RPB2 and the partial beta-tubulin gene from Sordariales, respectively. Molecular phylogenetic analyses were carried out with representative taxa from the three orders, and the taxonomic position of the new lineages were determined. Resynthesis experiments were also carried out with those isolates, and none of the isolates had a negative effect on the inoculated plants. All the fungi used for inoculation colonized the roots intraradically, and the Pleosporales and Magnaporthales isolates produced microsclerotica in the roots. Based on the phylogenetic analysis, the 33 root-endophytic isolates formed four well-supported clades within three orders (one in Magnaporthales and Sordariales, respectively, and two clades in Pleosporales). Our results indicate that all the new and recently described lineages are widespread endophytes in grasslands.

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The bZIP-type transcription factor AtfA orchestrates key cellular processes during asexual development in *Aspergillus nidulans*

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While the morphological aspects of conidiation are well characterized in filamentous fungi, the identity of the central transcriptional regulator integrating developmental and metabolic programs has remained elusive. To address this in Aspergillus nidulans, we conducted comprehensive transcriptomic and ChIP-seq analyses using conidia from wild-type, ΔatfA deletion mutant, and atfA: 3XFLAG-complemented strains grown under stress-free and menadione-induced oxidative stress conditions. Our integrated analysis reveals that AtfA serves as a master regulator coordinating asexual development, oxidative stress defense, and the underlying primary metabolic pathways. Notably, a substantial overlap in AtfA-bound and -regulated genes was observed in both untreated and menadione-exposed cultures, suggesting that developing conidia are transcriptionally pre-programmed for stress adaptation (Kocsis et al., 2023). AtfA directly regulates genes involved in diverse cellular processes, including HogA/SakA MAPK signaling, light-dependent control of sporulation, mitochondrial function, and eisosome biogenesis. It also governs the biosynthesis of trehalose and polyols, crucial for spore maturation and dormancy. Furthermore, AtfA exerts control over central carbon metabolism, including glycolysis, gluconeogenesis, and the TCA and glyoxylate cycles. Together, these findings position AtfA as a central transcriptional hub that links developmental morphogenesis with metabolic and stress-responsive gene networks, ensuring robust conidial formation and environmental resilience in A. nidulans. Kocsis et al. (2023) Cells 10, 463.

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Effect of Enterocin Ent7420 on intestinal microbiota, caecal enzymatic activity and health in broiler rabbits infected with a methicillin-resistant *Staphylococcus epidermidis* P3Tr2a strain

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Antimicrobial resistance (AR) is a global concern to humans, animals, and environmental health. Livestock can be a reservoir of different AR bacteria. Increasing occurence of methicillin-resistant staphylococci (MRS) in food animals leads to special attention, because of their difficult treatment and zoonotic character, posing a risk to human health due to contamination of animal products. Rabbits are important livestock animals because of their healthy and delicious meat. Rabbits are also very sensitive animals during the weaning period associated with increased stress and susceptibility to diseases, which adversely affects their health, growth and productivity. Current research is focusing on natural antimicrobial compounds to maintain animal health. The use of enterocins (Ent), antimicrobial protein substances produced by beneficial strains of the genus Enterococcus, belonging to postbiotics, could be an effective way to reduce/eliminate the MRS incidence in rabbits. Therefore, the aim of this study was to simulate the spoilage/pathogenic environment applying methicillin-resistant Staphylococcus epidermidis SEP3/Tr2a (MRSE) strain and to examine the effect of non-commercial Ents: EntA/P and Ent7420 on gut microbiota, caecal enzymatic activity (CEA), jejunal morphometry (JM), glutathione-peroxidase (GPx) and phagocytic activity (PA) in rabbits. Tested EntA/P and Ent7420 (50 µL/animal/day) and the MRSE strain (105 CFU/mL, 500 µL/animal/day) were applied to rabbits for 21 days, also in combination (EntA/P+MRSE; Ent7420+MRSE); achieved results were compared to control data (without any additives; negative control). The MRSE negatively influenced the JM, GPx and PA activity. During Ents application, the PA was stimulated in rabbits ($p<0.001$), also after their withdrawal (preventive effect). Higher CEA, improved JM parameters and the in vivo antimicrobial effect (reduced counts of the MRSE strain, staphylococci, enterococci and coliforms) of EntA/P and Ent7420 were noted during their application. Both Ents optimized also the negative effect of MRSE strain on GPx and JM (medicinal effect). Achieved results confirmed the beneficial effect of EntA/P and Ent7420 on immunity and the intestinal environment of rabbits and reflect their great potential as feed additives in rabbit nutrition to improve animal health with protective and medicinal effects.

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The effect of mundticin produced by the strain *Enterococcus mundtii* EM41/3 in horses breed Norik from Muráň

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Nowadays, breeders have been looking for the natural and effective strategies to maintain animal health. The same interest has occurred among horse breeders. Among natural components used for this purpose belong also bacteriocins. They represent antimicrobial substances of proteinaceous character, which were allotted in the group of postbiotics. It means, substances with beneficial effects for the host where they were used. The aim of this study was to find effect of postbiotic-bacteriocin (mundticin) produced by the beneficial autochthonous strain Enterococcus mundtii EM41/3 in the horses breed Norik from Muráň, which is a Slovak breed associated with the area in the central-east Slovak region. Partially purified substance Mundticin EM41/3 with activity 204 800 AU/ml was used in the dose of 100 µl for each horse; each horse serves as a control for itself. Thirteen mares of the Norik breed from Muráň of various ages were included in the experiment. Horses were healthy; they fed on hay or they were on pasture and/or located in a stall. Mundticin dose was applied in diet bolus, one per day for a duration 21 days. Sampling was provided at day 0/1, at day 21 and finally at day 42 (3 weeks after mundticin cessation). It was approved by the Slovak forest administration and the horses location head. Microbiological status was examined using standard microbiological methods and next-generation sequencing (NGS) as well. At day 21, reduction of coliforms (decrease from 0.76 up to 1.20 log cycle) and pseudomonads (0.76 log cycle) was noted. Bacterial reduction after mundticin application was also noted at day 21 NGS at different levels testing by NGS and comparing with day 0/1. Mundticin stimulated phagocytic activity (71.55 to 70.77), while GPx values were lower at day 21. Hematological profile and biochemical parameters were found in normal range values. Increase in hydrolytic activity, especially xylanolytic, was noted. Results indicated a progressive strategy for the use of postbiotics in horse breeding.

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Atypical Bacterial Pathogens in Healthcare-Associated Water Matrices

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Hospital water distribution systems are often overlooked as potential reservoirs for nosocomial infections. However, various water matrices including drinking and domestic hot water systems; water utilised in relation to air conditioning devices (e.g. cooling towers, humidifiers); water treatment equipment such as dialysis machines, RO systems, dental units and mechanical filters, as well as other sources like aqueous solutions or bottled water, may harbour opportunistic microbial contaminants. While drinking water is generally considered safe for healthy individuals, in healthcare environments (especially those treating immunocompromised patients) even low-risk water sources can be associated with waterborne nosocomial pathogens. The presence of Legionella species is a well-known indicator of premise plumbing-related risk, whereas Pseudomonas aeruginosa often indicates biofilm formation and local colonisation. However, in recent years, the water microbiology laboratory of the NCPHP has received multiple requests to investigate unusual hospital infections

suspected to be of water origin. This presentation highlights three case studies that revealed atypical bacterial infections originating from different water sources: Achromobacter sp. identified in water used for incubator humidification, two Ralstonia species colonising purified bottled water intended for the preparation of inhalation solutions and Delftia acidovorans isolated from tap water within a hospital ward. These cases demonstrate that, contrary to general belief, neither distilled nor RO-purified water is sterile and that water treatment devices usually deteriorate rather than improve microbial water quality. The use of non-sterile water in invasive procedures or for solution preparation may contribute to healthcare-associated infections caused by a broader range of microorganisms. These findings emphasize the need for periodic risk assessment of assumed "low-risk" water sources in clinical settings.

Characterisation Of Endemic Aspergillus Flavus Strains in In Vitro And Microplot Field Experiments

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Aspergillus flavus biocontrol relies on competitive exclusion through non-aflatoxin-producing (atoxigenic) strains, with commercial products like Afla-Guard and AflaSafe utilising this principle. Atoxigenic traits result from naturally stable, irreversible mutations, and both aflatoxigenic and non-aflatoxigenic strains typically cohabit without mutual inhibition under natural conditions. However, fungal interactions have been described inconsistently in the literature, creating uncertainty about the precise mechanisms underlying competitive exclusion and the effectiveness of aflatoxin reduction. We characterised the interactions between toxigenic and atoxigenic A. flavus isolates, both in vivo and in vitro. Detection of aflR, norA, and omtA genes was performed to distinguish strain types. We analysed colonisation capability, volatile compound effects, morphological differences, and correlations with aflatoxin B1 production. In vivo experiments involved inoculating corn ears under irrigated and non-irrigated conditions, using both soil and ear application protocols. Detection of aflR, norA, and omtA genes proved unsuitable for distinguishing atoxigenic from toxigenic A. flavus strains. Non-aflatoxigenic isolates contained high-impact mutations in aflatoxin gene clusters. Aflatoxin B1 production showed a negative correlation with increasing fungal biomass. Atoxigenic and toxigenic strains demonstrated equivalent colonisation capabilities without volatile compound effects in vitro. The toxigenic strain developed fewer aerial hyphae, but sclerotium production remained unchanged when interacting with atoxigenic strains. In corn ear inoculation experiments, atoxigenic strains reduced average aflatoxin B1 contamination primarily in non-irrigated areas, though the reduction was not statistically significant. Therefore, when atoxigenic strains were applied to soil and toxigenic strains to ears, significant aflatoxin B1 production reduction occurred specifically in non-irrigated plots. Thus, our findings demonstrate that the effectiveness of competitive exclusion depends on environmental conditions and application methods, rather than direct fungal antagonism. The significant reduction achieved through soil application of atoxigenic strains provides evidence for optimised biocontrol strategies, particularly under water-limited conditions where aflatoxin contamination poses the greatest risk.

Utilization of Betulinic Acid in a Synergistic Combination with Amphotericin B Against Clinically Important Fungi

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There is a need for new therapeutic approaches against invasive fungal infections as they are becoming more common in parallel with the growing number of antifungal resistant fungi making their treatment difficult. Amphotericin B (AmB) is still one of the most used antifungals due to its wide range of efficacy and the first line antifungal against Mucorales because of their intrinsic resistance to most azoles. Despite AmBs lipid formulations (e.g. complexed, liposomal and colloidal dispersed) it still exhibits side-effects. Betulinic acid (BA) is a naturally occurring derivative of betulin found in a broad species of plants. It has demonstrated noteworthy antimicrobial and antitumor effect, it is able to cross phospholipid membranes and has a similar core structure to ergosterol (ERG), a prevalent sterol component of fungal membranes. Yet its utilization as in combination with antifungal drugs or as an additional treatment has not been thoroughly investigated. Therefore, this study is focusing on the synergistic interaction of AmB and BA against clinically important pathogens such as Candida, Aspergillus, Scedosporium, Fusarium and Mucorales fungi. Our findings revealed a significant improvement in the antifungal efficacy of AmB when administered in combination even with minimal amounts of BA (0.125 μg/mL), which aligns well with clinically important serum levels. Combination with BA also allowed for the dose reduction of AmB to be 16-fold less. Although we observed a non-linear growth inhibition in case of higher concentration combinations which were ineffective that can be defined as the Eagle-effect. Our in silico molecular docking analyses suggest an improved antifungal activity in case of combined application via pore formation. The binding affinity of AmB towards BA is slightly greater compared towards ERG. The highest affinity was observed when both BA and ERG were present in simultaneous multiple molecule binding analyses. Our findings suggest the formation of mixed pores consisting of AmB, BA and ERG, resulting in an amplified antifungal effectiveness. Which may be attributable to BA's lipophilic nature and its cellular membrane permeation ability.

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The seroprevalence of West Nile virus in poultry in Croatia in 2024

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West Nile virus (WNV) is one of the most widespread flaviviruses that causes vector-borne and emergent zoonosis. In a natural cycle, WNV is a pathogen maintained in a natural mosquito–bird–mosquito transmission cycle. Poultry, horses, and humans are accidental hosts with low and short-term viremia, which are not able to spread the disease and represent a source of infection. In most accidental hosts, including poultry, the infection is asymptomatic or with mild clinical signs. However, their suitability as sentinel animals lies in their rapid seroconversion with detectable antibodies shortly after infection. The first serologically confirmed case of WNV infection in poultry in Croatia was detected in 2013 in the northwestern part of Croatia. Since then, poultry has been continuously serologically tested for the presence of WNV IgG antibodies and serological studies have shown continuous circulation of WNV in poultry with IgG seropositivity ranging from 1.8% to 22.9%. During 2024, a total of 3,404 sera were collected and tested from 221 flocks of chickens and turkeys. The

poultry were older than three weeks and hatched during the year under investigation, which provides a reliable insight into current viral activity, while excluding the presence of maternal antibodies and antibodies developed in previous seasons. The overall seroprevalence of individuals was 10.8% and was similar in chickens (10.6%) and turkeys (11.6%). The analysis of seroprevalence at the flock level provides a significantly more objective insight into the actual distribution of viral activity in Croatia. The seroprevalence at the flock level was 40.3% and was significantly higher in turkeys (53.5%) compared to chickens (38.3%). Out of a total of 17 surveyed counties, no seropositive poultry flocks were detected in only three counties, but it is important to note that a smaller number of samples were analyzed in these three counties. The highest proportion of seropositive flocks was found in Virovitica-Podravina County (83.3%), followed by Osijek-Baranja County (77.1%), Zagreb County (53.3%) and Vukovar-Srijem County (52.4%). We also compared seroprevalence in extensively and intensively raised poultry, but the observed differences were not statistically significant. Seroprevalence in domestic poultry indicates the circulation of WNV in most counties during 2024, with higher seroprevalence in counties with increased mosquito population density and in continental Croatian counties.

Can photosynthetic green microalgal cultures help reduce the negative effects of salt stress on plants?

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The high natural salinity of soils and the insufficient or excessive use of fertilisers are causing soil degradation in many parts of the world, with negative impacts on agriculture. Soil inoculants based on algae or bacteria can provide a solution for sustainable agriculture in salt-affected areas. In our study, we investigated the effects of two microalgal cultures – Desmodesmus obliquus strain (KH/1), Chlorella variabilis/C. sorokiniana (KH/2) – on salt stressed crops. The two algal strains were isolated from Lake Balaton (KH/1) and Széchenyi thermal baths (KH/2) and identified. Our previous model experiments have shown that photosynthetic activity of plants is significantly reduced even after 30 min of 200 mM and 300 mM NaCl treatment. In the present experiment, Arabidopsis thaliana L. (ecotype Columbia-0) plants were grown under control conditions for 28 days. Four different treatment and irrigation groups were established: 1) the untreated or control group was irrigated with bidistilled water, 2) the salt-treated group with 100 mM or 200 mM NaCl solution, 3) the algae-treated group with algal culture or lyophilized algae, and 4) the salt+algae-treated group with 100 mM or 200 mM NaCl algal culture or lyophilized algae. After treatment, non-invasive photosynthetic activity measurements were performed daily on plants for 20 days. The values obtained showed a significant decrease in Qy light and Qy dark values characterizing the photosynthetic electron transport chain function under salt stress. When plants were treated with both salt and KH/1 algal cultures, these values were similar to the control, i.e. the photosynthetic activity values Qy light and Qy dark were not or only slightly decreased. However, when plants were treated with KH/2 algal biomass or lyophilized KH/2 algal biomass in combination with the salt treatment, these values clearly decreased but were higher than in the salt-treated plants. We can thus conclude that the green algae cultures of D. obliquus KH/1 strain and C. variabilis/sorokiniana KH/2 strain help plants to tolerate salt stress to different extents.

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Wastewater-based epidemiological surveillance of respiratory pathogens

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Wastewater analysis could provide insight into the general health status of the population. As a number of human pathogens are shed through faeces and urine, their genetic material can be detected at the wastewater treatment plant. Objective of wastewater-based epidemiology (WBE) is to assess the presence and quantify the concentration of a certain pathogen in sewage to complement existing public health surveillance systems. It can provide a large amount of information on the spatial and temporal spread of epidemics with high precision. Since July 2020, the NCPHP has been operating the Hungarian wastewater surveillance system, focusing on pathogens of public health relevance, including SARS-CoV-2, influenza A and B, as well as respiratory syncytial virus subtype B (RSV-B). 23 samples are collected on a weekly basis from wastewater treatment plants located in the capital and 18 county seats, representing approximately 40% of the Hungarian population. The samples are concentrated by ultrafiltration. After nucleic acid extraction, dPCR is used for the measurement of influenza A, RT-qPCR for Influenza B and RSV-B. Influenza A has been measured weekly since January 2023, and RSV-B since January 2024. Influenza B detection was included from the 48th week of 2024 to the 20th week of 2025 in accordance with the higher incidence rate published by the Sentinel Surveillance System of the NCPHP. Adaptation of influenza B reaction into existing dPCR multiplex reaction (beside SARS-CoV-2, and influenza A) is in progress. Whole genome sequencing of the positive influenza samples is also in the method development status. Data from wastewater potentially supplement the sentinel surveillance system. Using WBE, the course of an epidemic could be traced or in case of certain pathogens (e.g. Influenza A and B) even predicted.

Deciphering a versatile pathogen: quest for effectors and potential mobile unites in '*Candidatus Phytoplasma solani*' genomes

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During the co-evolution of microbial pathogens and their hosts, pathogens acquired a wide array of effectors, powerful molecular weapons that increase their virulence by disturbing hosts' developmental processes and defence mechanisms. Phytoplasmas (genus 'Candidatus Phytoplasma') encompass a group of uncultivable endocellular wall-less bacteria affecting numerous plant species and causing significant damage in agriculture worldwide. They have a dual parasitic cycle colonizing plant phloem and insect cells. Their genomes are small, repetitive and prone to rearrangements harbouring putative transposon-like elements named potential mobile units (PMUs). The aim of our research was to perform MiSeq and ONT sequencing and genomic studies of three 'Ca. P. solani' strains in order to get insight into the genomic diversity of this species and repertoire of putative effectors and PMUs. Furthermore, we aimed to investigate the influence of selected effectors on Arabidopsis thaliana plants and possible interactions with plant transcription factors. Comparative genomic studies on obtained genome drafts identified 38, 22 and 20 putative effector genes, respectively, including homologues of already characterized phytoplasma effectors. It was shown

that some of the predicted effector genes could be considered as species- or strain-specific ones. Frequently, putative effector and secreted protein genes were found within PMU-like regions. High level of genomic diversity among 'Ca. P. solani' strains was also found, suggesting rapid evolution of this species, which may contribute to its wide host range and adaptability potential. Our functional genomic studies on transformed A. thaliana plants overexpressing effector genes SAP11-like or SAP54-like have shown that SAP11-like and SAP54-like effectors significantly affected plant growth and development by inducing changes such as reduction of leaf size and leaf crinkling, reduced biomass, excess of axillary branches, changes in siliques and appearance of hairy leaves and stems. Moreover, in planta bimolecular fluorescence complementation (BIFC) assays detected interactions of SAP11-like effector with plant transcription factors AtTCP2 and AtTCP4, while SAP54-like effector interacted with AP1 and SEP3. This study provides novel data on diversification of 'Ca. P. solani' genomes and their interactions with host targets, which could facilitate deciphering the pathogenicity strategies of this successful and versatile pathogen.

Responses of endophytic communities in plants to nutrient changes

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Endophytic bacteria play important roles in plant growth and development. However, little is known about the regulation of nutrient status on plant endophytic bacterial community and their possible functions in relation to plant growth and development. Herein, a series pot experiments and field experiments were devised to examine the effects of essential element (P) and beneficial nutrient (Se) on the growth and quality of rice seedling/grass/tea plant, as well as the composition, diversity and potential functions of leaf endophytic microbiota. The results showed that at initial stage of P deficiency, the rice roots hosted significantly more endophytic bacteria belonging to Proteobacteria with putative plant growth-promoting functions. However, when the seedlings grew for 21 days under P deficiency conditions, the seedling growth was severely restricted, and Firmicutes with putative functions of detoxification became the predominant root endophytic bacteria. Although Se is not essential element for plant growth, Se amendments could enhance the growth and quality of H. compressa grass and tea plant, and also enriched endophytic bacteria potentially involved in metabolic pathway of amino acid, carbohydrates, coenzymes, as well as the bacterial endophytes with putative functions of environmental adaptation, energy metabolism and xenobiotics biodegradation. When the rice plant is subjected to different levels of P deficiency, as a response, the rice roots might selectively recruit endophytic bacteria to serve the plant growth according to their demand. Foliar Se spray not only improve the growth and quality of grass/tea, but also synergistically regulate the plant growth and quality via assembling the endophytic bacteria.

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Phylogenomics and taxogenomics in yeast research

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Thanks to increasingly cost-effective genome sequencing methods, a rapidly growing number of whole-genome sequences are becoming available in public databases. Due to the high number of sequenced microbial genomes, it has become possible to reconstruct the evolutionary history of groups of microbial species (phylogenomics). Combination of the phylogenomic analysis with phenotypic and ecological information improves species identification and classification (taxogenomics). Methods developed for inferring evolutionary and taxonomic relationships from genomic data can also be applied to yeasts. One possibility is the comparison of entire genome sequences by ANI (Average Nucleotide Identity) and GGDC (Genome-to-Genome Distance Calculator). Another strategy is the calculation of genomic metrics from the comparison of the sequences of (trimmed and concatenated) orthologous genes or their translated products (proteins) shared by all genomes involved in the analysis (e.g. AAI: average amino acid identity of the orthologous proteins, POCP: percentage of conserved proteins, PAPO: presence-absence patterns of orthologues). Phylogenetic trees can be inferred from aligned concatenated orthologous sequences ("supergenes") or by coalescence analysis. All these methods are challenged by the structural heterogeneity of genomes. For example, many yeast strains are alloaneuploids or allopolyploids or have chimeric genomes consisting of segments of different phylogenetic histories. This structural complexity remains hidden when a haploid consensus genome sequence is assembled from the sequencing reads (e.g. by collapsing heterozygous sites into consensus sequences). To overcome this issue, haplotype phasing of alleles (assigning them to subgenomes) is needed for correct inference of phylogenetic relationships of non-haploid strains. Another problem is caused by the frequent diversity of conspecific genomes. A possible solution to this problem is pangenome analysis. A pangenome captures the complete genetic diversity within a species; it shows all versions of the segments that are diverse in the genomes of conspecific strains. Both haplotype phasing and pangenome analysis require sophisticated bioinformatics tools and are therefore not yet widely used in the phylogenetic and taxonomic analysis of yeasts. The potential and limitations of the above-mentioned strategies are demonstrated on a heterogeneous group of yeasts,

Emerging and re-emerging viruses in solanaceous and cucurbitaceous crops of Croatia

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Over the years 2021-2024 a large number of vegetable samples was tested for viruses from greenhouse and open field cultivation by targeted immunostrips and RT-PCR. The sampling process focused on plants exhibiting obvious and diverse virus-like symptoms, particularly those observed in leaves or fruits. This preferential sampling strategy aimed to maximize the chance of detecting and studying plant viruses by targeting plants that displayed noticeable signs of infection. Greenhouse grown tomatoes from three continental and one coastal site, peppers from three continental sites, as well as cucumbers and zucchini from several continental sites were included in the study. A smaller subset of representative samples was subjected to non-biased virus detection and identification by high throughput sequencing (HTS) on Oxford Nanopore Technologies (ONT) and Illumina platforms. Besides viruses adversely affecting tomato, pepper and cucurbits like cucumber mosaic virus (CMV),

tobacco mosaic virus (TMV) and potato virus Y (PVY), a high incidence of tomato spotted wilt virus (TSWV) was recorded in tomato and pepper. Even though most of those tomato and pepper cultivars are marketed as having intermediate or high resistance to the virus, recorded symptoms were very severe and reminiscent of emerging TSWV resistance breaking (RB) strains. Nonetheless no TSWV-RB strains have been recorded so far. On the other hand, PVY-NTN strain was often found in mixed infection with TSWV, and some other viruses, in those samples. Potato virus S (PVS) was found for the first time in the country in one location in tomatoes together with PVY-NTN. Cucurbits were largely affected by viruses already recorded in Croatia like potyviruses PVY, watermelon mosaic virus (WMV), zucchini yellows mosaic virus (ZYMV) and sporadically by CMV (cucumovirus). It is noteworthy that an emerging strain of WMV and emerging Moroccan watermelon mosaic virus (MWMV) were recorded for the first time in zucchini with completely unmarketable fruits. Interestingly, no emerging geminiviruses have been recorded. Besides the molecular characteristics of emerging and re-emerging viruses in the vegetable viromes, their potential impact on the production and the need to improve the vegetable disease management will be discussed.

INVESTIGATION OF SUCCINATE DEHYDROGENASE INHIBITOR (SDHI) FUNGICIDES RESISTANCE IN GRAPE POWDERY MILDEW (ERYSIPHE NECATOR) POPULATIONS IN EGER VINEYARD, HUNGARY

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Powdery mildew is one of the most common fungal diseases associated with grapevine (Vitis vinifera) caused by the obligate biotrophic fungus Erysiphe necator. The continuous and intensive use of succinate dehydrogenase inhibitor (SDHI) fungicides results in a gradual development of sensitivity by alterations in several genes. The occurrence of resistant E. necator populations are becoming more common and it has been previously shown that pathogens with reduced sensitivity to one certain fungicide could be still sensitive to other fungicides. The main goal of our work was to detect point mutations in genes causing fungicide resistance and to describe fungicide sensitivity of Hungarian populations of E. necator in the vineyard of Eger. The sensitivity of the tested grape powdery mildew isolates were tested for two common SDHI fungicides. In the experiment, the cell culture plate containing aqueous agar contained the given fungicide in a specific concentration, on the surface of which powdery mildew conidia were spread with the help of a sterile brush. The fungicides used were tested in a total of five different concentrations. The tested SDHI fungicides were fluxapyroxad and fluopyram. Following 24 hours of incubation, the ratio of germinating and non-germinating spores and were determined. The germination rates were compared to the control (conidia spread on water agar without fungicides) germination rate. Out of the two tested SDHI fungicide, reduced sensitivity was observed in case of the fluxapyroxad. In addition to in vitro resistance tests, point mutations associated with amino acid substitution (SDHB-p.H242R; SDHC-p.G25R; SDHC-p.G169D) was mapped by gene sequence analysis in the related genes (sdhb and sdhc). Based on our results, the reduced sensitivity in case of the fluxapyroxad is consistent with the presence of the SDHC-p.G169D amino acid substitution. In our further studies we would like to expand the list of tested fungicides with other SDHIs used in the viticultural practice.

Multiplex PCR-based genotyping of *Fusarium* isolates for predicting mycotoxin contamination

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Estimates indicate that up to 80% of the global grain supply is affected by mycotoxin contamination, of which 5–10% is considered risky for human or animal consumption due to the potential for severe acute or chronic health effects. Fusarium species are among the most significant mycotoxin-producing filamentous fungi. The trichothecenes (e.g. deoxynivalenol – DON, T-2 toxin), zearalenones (ZEA), and fumonisins (FB1, FB2) that they produce are classified among the most hazardous mycotoxins not only because of their biological effects but also because of their high incidence in agricultural products. The biosynthesis of these complex-structured mycotoxins frequently involves between 5 and 30 different genes, many of which are co-expressed. The presence of specific genes encoding key enzymes is essential for the functioning of the biosynthetic pathway. The objective of the present study was to detect the presence of essential genes involved in the biosynthesis of major mycotoxins in a collection of previously characterised, mycotoxin-producing Fusarium isolates using molecular biology techniques. In addition, a multiplex PCR approach was designed and refined to assess the toxigenic potential of these isolates. The long-term objective of this research is to develop a method that can be used in the field for screening and characterising Fusarium strains based on their capacity to produce mycotoxins.

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Virulence-associated traits in *Mucor lusitanicus*

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The Mucorales order comprises highly resilient fungi responsible for mucormycosis, a lethal and increasingly prevalent angio-invasive infection, ranking as the third most common invasive fungal disease. Spore coat (CotH) proteins are critical virulence determinants, mediating adhesion, colonization, and tissue invasion, and regulating spore germination and structural integrity. We performed a phenotypic and functional analysis of Mucor lusitanicus CotH-deficient mutants generated via CRISPR-Cas9 to investigate their roles in cell wall architecture, viability, and pathogenicity. Fluorescent staining and TEM revealed that cotH gene deletions altered inner spore coat structure, spore size distribution, and hyphal morphology. ΔcotH12 showed atypical septation and aberrant spore wall formation, while ΔcotH9 displayed features of programmed cell death, including vacuolization, cytoplasmic disorganization, and fragmentation, highlighting its role in spore and hyphal viability. Loss of CotH6 caused abnormal sporangial morphology and reduced sporulation. Calcofluor white staining indicated increased fluorescence in ΔcotH4, suggesting elevated chitin content or structural rearrangements enhancing dye accessibility. Functional assays revealed differential stress susceptibility. Virulence testing showed host- and strain-specific effects: ΔcotH9 and ΔcotH12 were attenuated in Drosophila melanogaster, whereas ΔcotH3 and ΔcotH4 exhibited reduced pathogenicity in diabetic ketoacidosis (DKA) mice, correlating with elevated GRP78 expression. These results demonstrate that CotH proteins have non-redundant roles in morphogenesis, spore wall biogenesis, stress adaptation, and host interaction, supporting their potential as diagnostic markers and therapeutic targets in mucormycosis.

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Arbuscular Mycorrhizal Fungi in Enhancing Plant Stress Tolerance

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Due to global climate change drought and heat are becoming increasingly severe, reducing the yield of many crops, including tomato. In addition to conventional agricultural practices, the use of symbiotic microorganisms such as arbuscular mycorrhizal (AM) fungi is emerging as a promising approach to improve plant stress resilience. In this study, we evaluated the impact of seven different AM fungal strains on the drought and heat stress tolerance of tomato. The results demonstrated variation in plant stress tolerance, which was linked to the functional diversity of the AM fungal strains. Among the fungi tested, Funneliformis mosseae and Septoglomus constrictum proved most effective in alleviating biomass loss, sustaining photosynthetic performance, and preserving redox balance by boosting antioxidant enzyme activity and promoting proline accumulation under both drought and heat stress, as well as drought and heat shock. Consequently, these two strains were chosen for more in-depth analysis in follow up experiments. F. mosseae inoculation reduced expression of HSP70 and HSFA2 genes and decreased MDA levels indicating diminished stress effect. Phosphorus content of shoots also increased, most notably under combined stress, followed by water deficit and then heat stress. In parallel, the expression of several phosphate transporter genes was upregulated under stress conditions, suggesting that F. mosseae induces enhanced phosphate transporter activity, which may contribute to stress alleviation. The specific effect of S. constrictum strain on tomato heat stress response was examined using transcriptomic and bioinformatic approaches. Short-term intense heat treatment elevated H_2O_2 levels indicated the presence of stress, which was significantly less pronounced in mycorrhizal plants. Functional analysis of differentially expressed genes revealed enhanced metabolite mobilization between root and shoot in mycorrhizal plants, as well as the activation of hormonal signalling pathways – particularly auxin and jasmonate – induced by the AM fungus under heat stress.

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Syngas fermentation

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Rapid advances in technology and the expanding knowledge base are creating significant opportunities in the renewable energy sector. Anaerobic fermentation is among the most promising approaches, converting organic biomass into alternative energy carriers, primarily methane (CH_4). Recent developments have not only improved CH_4 yields but also expanded the range of substrates. Beyond organic matter, gaseous substrates such as hydrogen (H_2), carbon monoxide (CO), and carbon

dioxide (CO_2)—the main components of syngas—can also be metabolized by methane-producing microbial communities. Syngas, a mixture primarily composed of H_2, CO, and small amounts of CO_2, is typically produced via gasification of biomass, agricultural residues, or organic waste. It serves as a versatile intermediate linking renewable feedstocks to a range of sustainable products, including biofuels, electricity, and green chemicals. In anaerobic biogas fermentation, syngas can be biologically converted into CH_4 by methanogenic Archaea and carboxydotrophic (CO-reducing) bacteria. The two main carboxydotrophic pathways are hydrogenesis ($CO + H_2O \rightarrow H_2 + CO_2$) and acetogenesis ($4CO + H_2O \rightarrow CH_3COOH + 2CO_2$). The resulting CO_2, H_2, and CH_3COOH are subsequently utilized by acetotrophic (acetate-fermenting) and hydrogenotrophic (H_2-oxidizing, CO_2-reducing) methanogens, further enhancing CH_4 production. Our main purpose was the determination of the metabolic activity of the mixed anaerobic biogas forming community during the feed of various composition ratio of the Syngas based gas mixture both in short (3 days) and longer term (30 days), while monitoring the microbial composition (metagenomic) changes. The proposed strategy opens new pathways for waste valorisation, decentralized renewable energy generation, and carbon recycling. Bio-methanation of syngas supports the circular economy concept and contributes to reducing reliance on fossil-based natural gas by producing high-purity biomethane suitable for grid injection or use as a transportation fuel. Deep understand of the metatranscriptomic and metagenomic changes escalated via the Syngas utilization could lead to more efficient and sustainable fermentation systems.

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The Impact of the Yap1 Transcription Factor on the Virulence of *Candida auris*

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Candida auris is a multidrug-resistant yeast first identified in Japan in 2009 and now considered a global health threat. Difficult to identify with standard methods, it persists on surfaces, spreads rapidly—especially in immunocompromised individuals—and often resists multiple antifungals, with mortality rates of 30–60%. Its distinct clades (e.g., South Asian, African, Iranian) are globally distributed and vary in resistance profiles. Clinically, C. auris is linked to candidemia, wound and urinary tract infections, otitis media, and occasionally meningitis or myocarditis. In the bloodstream, oxidative stress is partially counteracted by the transcription factor Yap1. This bZIP factor, known in S. cerevisiae as a key regulator of oxidative stress responses, was deleted in C. auris strain B11220 (Tokyo isolate) in our study. Deletion was PCR-confirmed, and mutant phenotypes were validated by complementation. We used qPCR to measure the expression of several Yap1-regulated genes (e.g., GLR1, SOD1, SOD2, TRR1, TSA1, GSH1, GSH2) in wild-type and mutant strains after tBOOH (tert-Butyl hydroperoxide) exposure. The most notable expression difference was in TRR1, favoring the wild type. Both strains were tested under various stress conditions: oxidative, heavy metal, osmotic, cell wall stress, iron depletion, and iron overload. No phenotypic differences were seen with MSB (menadion sodium disulfite), Congo Red, NaCl, sorbitol, or $AgNO_3$. However, the mutant showed increased sensitivity to tBOOH, H_2O_2, NaOCl, diamide, $CdCl_2$, and heat. While iron overload had no effect, DFP (deferriprone)-induced iron deprivation significantly increased mutant mortality. We further assessed pathogenicity in a mouse model, biofilm formation on human skin, and macrophage-mediated immune responses. Although infectivity was reduced in the mutant, biofilm formation and phagocytic uptake were similar between strains. Finally, macrophages and dendritic

cells exhibited greater cytotoxicity toward the mutant, aligning with its increased susceptibility to intracellular oxidative stress.
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Mapping the microbial dark matter – relevance of *Patescibateria* in petroleum hydrocarbon contaminated aquifers

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Even though the effort of environmental microbiologist, only slight number of bacteria can be thoroughly investigated in such complex environments like groundwater and soil. One of the prime examples is Patescibacteria, a newly proposed phylum of bacteria, formerly member of the Candidate Phyla Radiation (CPR). This group of bacteria have been found to exhibit remarkable diversity and distribution in wide range of environments. On the other hand, as they have a small genome and cell size coupled with restricted metabolic capacities, they are considered to thrive only in a symbiotic lifestyle. Our investigation focused on a peculiar environment, we studied the diversity and distribution of Patescibacteria in two petroleum hydrocarbon contaminated aquifers in Hungary. The bacterial communities were investigated by cultivation-free molecular methods such as traditional and Patescibacteria-specific 16S rRNA gene amplicon analysis, furthermore, via genome-resolved metagenomics, and metagenome-informed microscopy approaches in order to identify the potential host of symbiotic Patescibacteria. At the contaminated sites we identified two groundwater wells with microbial communities showing high Patescibacteria abundance. The results obtained shed light on the fact that the traditional 16S rRNA gene specific amplicon primers tend to underestimate both the diversity and the abundance of Patescibacteria. By using an alternative primer set designed for the better detection of Patescibacteria, increased abundance was revealed in cases of Ca. Shapirobacteria, Ca. Woesebacteria, Ca. Kaiserbacteria, Ca. Moranbacteria and Saccharimonadaceae. By using genome-resolved metagenomics, we managed to show that in one of the groundwater wells members of the Patescibacteria were the most dominant community players, followed by Gammaproteobacteria and Desulfobacterota. Besides, we managed to reconstruct the 1.45 MB large genome of a Patescibacteriales bacterium, most closely related to Ca. Falkowiibacteriota. Additionally, an integrated CARD-FISH and SEM approach is proven to be an adequate tool for visualizing cell-to-cell interactions in environmental microbiology.
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Aspergillus flavi and *nigri* concentrations in outdoor air in Hungary – preliminary data of a 16-year-long observation

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Due to their mycotoxin production, Aspergillus Flavi (AF) and Nigri (AN) have a tremendous impact on food safety. However, their role in allergy and mycoses is also a main concern. These fungi produce large amounts of airborne spores for colonization of crops, postharvest products and a wide variety of organic materials. Despite that, no long-term observations are available in the literature about their airborne concentrations. This study aims to analyze long-term concentration data of AF and AN from outdoor air in Hungary. Air samples were taken by an Andersen-type sampler (100L) in random outdoor locations in Hungary (66% in Budapest) between 2009 and 2024. Samples were impacted onto malt extract agar with 2% chloramphenicol and incubated at 25°C for five days. Airborne fungal colonies were identified by morphological characteristics using a light microscope at 400× magnification. The colony-forming units were counted, and the concentrations (CFU/m3) were calculated using the 'positive hole correction' method. In our preliminary analyses, 238 air samples were used to calculate the yearly sum of total fungal taxa (TF), AF, AN and Cladosporium spp. (CL, the predominant airborne taxa in Hungary). The ratio of AF and AN of the total fungal count (AF/TF% and AN/TF%) was calculated, as well as the rate of Cladosporium count (AF/CL and AN/CL). The association between aerobiological and meteorological data measured in Budapest was assessed: drought index PADI0 and FAI, summer average (avg) temperature (SAT) and avg temperature of the period April to August, yearly total and summer precipitation were taken into account. The 15-year average (2009-2023) of relative abundances was low, <1.0% (AF/TF%=0.1, AF/CL=0.0013, AN/TF%=0.52 and AN/CL=0.0091). A dramatic increase of AF/CL (68-fold) and AN/CL (20-fold) was observed in 2024, when extreme Aspergillus concentrations were measured in urban air samples (AF>3000; AN>5000 CFU/m3). The 15-year average of the drought index FAI was 8.31 (st.dev. 3.38) and was the highest (>10) in four years (2009, 2013, 2022 and 2024). In 2009, 2022 and 2024, the values of AN/TF% were relatively high (>1.0). The 15-year average of SAT was 22.56°C and reached a maximum in 2024 (24.67°C) when AF/TF% was also the highest (5.23). In recent years, the rapid increase in the concentrations of AF and AN in Hungarian air was probably induced by climate change. The investigation and prevention of the related health risk is a necessary future task.

Open-field trials of mycorrhizal and bacterial inoculation on yield and soil parameters in sunflower, maize, and pepper production

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Sunflower (Helianthus annuus L.) and maize (Zea mays L.) are globally significant crops of economic importance in agriculture. Pepper (Capsicum annuum L.) constitutes one of the most commercially valuable vegetable crops cultivated worldwide. However, modern agricultural challenges, including climate change, soil degradation, water management, and the use of synthetic chemicals, such as pesticides and fertilisers, make alternative solutions necessary to improve productivity besides cost effectiveness. This study aims to investigate an environmentally friendly and sustainable variation through the application of a commercial product containing mycorrhizal fungus and beneficial soil bacteria, Azospirillum sp., in field conditions. Randomised complete block design open-field experiments were established on brown clay soil and sandy soil in Hungary. Instead of the usual

technology, we carried out the application by creating a layer containing the inoculant on the surface of the seeds. In the examination of the three types of target plants, in addition to crop yield, the nutritional analysis of the leaves and soil characterisation, including chemical and some microbiological parameters were investigated. Plate diameter for sunflower was also recorded, moreover, harvested peppers were measured for berry weight, stalk weight, rate of red and green fruits, dry matter content, capsaicin content and values of pepper colour (ASTA). The applications of these beneficial microorganisms together have a significant increase in the yields of sunflower and maize. Although, the difference between treated and untreated peppers was only visually apparent during the first month, the treatment did not result a significant increase in yield, but it did improve the quality parameters of the crop and the efficiency of nutrient use. It should be noted that the capsaicin content of the pepper did not change significantly. Soil analysis showed enhanced nutrient availability, particularly phosphorus in maize and nitrogen in sunflower. These findings indicate that applied microbial product can enhance both crop productivity and quality through enhanced nutrient uptake efficiency, but the effectiveness of the treatment varies according to soil type and target crop. Our preliminary results may help to expand the possibilities for applying mycorrhizal and beneficial soil bacteria inoculants and increase our knowledge under open-field conditions.

Species distribution and antimicrobial susceptibility of dermatophytes isolated at the University of Debrecen

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Dermatophytoses are among the most common fungal infections globally, affecting an estimated 20–25% of the global population. Because they are not life-threatening, these infections have often been overlooked. However, recent changes in their epidemiology and drug resistance patterns have drawn more attention—especially with the worldwide spread of Trichophyton indotineae, which is increasingly linked to terbinafine-resistant cases. In 2023, our laboratory began routine culturing and presumptive identification of dermatophytes from skin and nail samples. We collected 51 independent isolates, with 45 available for antifungal susceptibility testing. Isolates were identified using Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry with the Bruker Filamentous Fungi database and MSI-2. Susceptibility testing was performed according the European Committee on Antimicrobial Susceptibility Testing broth microdilution methodology for dermatophytes to fluconazole, itraconazole, voriconazole, terbinafine, and amorolfine. The most common species was Trichophyton rubrum (53%), followed by the Trichophyton mentagrophytes complex (24%) and Microsporum canis (8,8%). One isolate of Trichophyton indotineae was found in a patient who was not a Hungarian national. All nail samples yielded Trichophyton rubrum, while skin samples showed more species variation. All isolates tested were wild-type in terms of susceptibility to all the antifungal agents tested, where Epidemiological Cut-off Value available. These findings suggest that antifungal resistance is not currently an issue in our patient population. However, the detection of Trichophyton indotineae in Hungary is noteworthy and highlights the need for continued monitoring. Early identification and awareness among labs and clinicians will be key in preventing the spread of resistant strains.

The in vitro interaction of deferiprone with antifungal agents against *C. auris*

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Candida auris is well-known for its poor susceptibility to conventional antifungals, which warrants examination of novel approaches. Limiting the availability of micronutrients, including iron, is a promising antimicrobial strategy and proven to enhance the activity of various antimicrobial agents in vitro. Therefore, we have evaluated the activity of deferiprone, a clinically available iron chelating agent in combination with echinocandins, amphotericin B and fluconazole against a panel of C. auris strains including three echinocandin resistant ones with defined FKS mutations representing the four major clades. Minimal inhibitory concentrations (MICs) were determined according to CLSI M27-A3 guidelines, and drug interactions were assessed using checkerboard microdilution assays at 24 and 48 hours. Interactions were analyzed using both fractional inhibitory concentration indices (FICIs) and the Bliss independence model. Synergy was most observed for echinocandins, while interaction was generally additive or indifferent for fluconazole and amphotericin B, except for Clade II. Antagonistic interaction was only observed in one case. Our results suggest that iron chelation may enhance the activity of echinocandins against C. auris in a clade and isolate specific manner.

Effects of VOCs released by endophytic bacterial strains isolated from the raspberry cane midge and the raspberry slender ornamental beetle on elderberry shoot buds in vitro

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According to the Holobiont concept, the bacteria found in plant leaf, stem and root tissues are growth-promoting bacteria living in and on higher plants, due to the VOCs (Volatile Organic Compounds) they emit. Therefore, we investigated the effect of endophytic bacterial strains isolated from the cocci and larvae of the raspberry (Rubus idaeus) budworm (Thomasiniana theobaldi) and the raspberry slender ornamental beetle (Agrilus aurichalceus) on the growth of Sambucus nigra shoot plants, physically separated from each other but in a common gas chamber. We measured the plant biomass increment, hence the induction capacity of putative VOC compounds, as well as the VOC compounds released by SPME-GC-MS analytical method. Bacteria were isolated from raspberry cocci and larvae under surface disinfected conditions and identified by 16S rRNA gene sequence analysis. Bacterial cultures propagated in petri dishes were placed in vitro in the micropropagated elderberry shoots without inoculation and hermetically sealed. After 30 days, the positive or inhibitory effects of the bacterial strains were examined: plant size, multiplication rate, size of the propagules, possible callus formation on the shoots. Our results show that of the 20 or so strains isolated, we observed light green leaf blade and slight callosity with strain MG/H2, with many lateral shoots. Strain DL/5/2 caused yellowish, juvenile leaves with a more fragile main stem and more callus. The MG/P2 strain effected more vivid greenish leaf blade on the shoots, but the main stem was significantly elongated compared to the side shoots. In strain MG/P3, we observed a high degree of shoot formation and many lateral shoots. Here the rate of growth was significantly measurable. With DL/5/1/2 strain, we observed rapidly senescing lower leaves with high callus formation and

moderate reproductive capacity. Strain DL/6/1 had a significantly larger stem part of the original main shoot, as confirmed by the measurement of plant biomass. It can be concluded that strain DL/6/1 and MG/P3 proved to be the most efficient for the release of plant growth stimulating VOCs, while the inhibitory VOC release of strain DL/5/2 was surprising. Gas chromatography analyses of gas samples taken from the atmosphere of in vitro cultures were performed to confirm the VOCs emission results, which are ongoing, but preliminary results indicate that a large number of VOC compounds were isolated and detected.

Evaluation of antibiotic resistance in *Riemerella anatipestifer* strains derived from poultry in Hungary

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Riemerella anatipestifer is a Gram-negative, non-motile, non-spore forming, encapsulated rod-shaped bacterium. This facultative pathogen is present in every country where ducks and geese are kept under intensive conditions, and has a considerable economic impact as it can cause severe losses in the populations (10-75%). The primary treatment of R. anatipestifer infection is antibiotic therapy, even though the pathogen has acquired resistance to several commonly used antibiotics. Vaccination is an option, but as there is no cross-protection among the 25 known serotypes, it has to be tailored to the prevalent bacterial serotypes in the population. The aim of our study was to evaluate the antibiotic susceptibility of 46 strains of R. anatipestifer collected from different regions of our country. Following the CLSI VET01S guideline (2004), we determined the minimal inhibitory concentration of enrofloxacin, in the concentration range of 0.03 µg/ml to 16 µg/ml, tetracycline (from 0.06 µg/ml to 32 µg/ml), erythromycin and doxycycline (both in the range of 0.125µg/ml to 64 µg/ml), and our future plans include expanding the tested antibiotic panel with other drugs currently being used in the poultry industry. Our results showed that doxycycline demonstrated the highest activity with MIC50/MIC90 values of 0.25/0.5 µg/ml, followed by tetracycline (2.0/8.0 µg/ml) and enrofloxacin (4.0/8.0 µg/ml), while erythromycin showed the lowest activity with MIC50 values of 64.0 µg/ml, and the MIC90 value being higher than 64.0 µg/ml. According to our data, 89% of the strains are resistant to enrofloxacin, 73% are resistant to erythromycin, while in case of doxycycline and tetracycline, none of the strains showed MIC value above the 16 µg/ml breakpoint. We have very little information regarding the genetic background of antibiotic resistance of R. anatipestifer in Hungary. Thus, the genomic DNA of the strains was isolated and will be sent for whole genome sequencing in order to acquire deeper insights into the diversity of the resistance-associated genes, the virulence factors and the mobile genetic elements among strains. Using bioinformatic prediction tools, core genome analysis of these strains may uncover antigens suitable for the development of novel subunit vaccines. Therefore, our results may contribute to the implementation of protective measures such as the optimal treatment strategy and vaccine development against R. anatipestifer.

In vivo efficacy of rezafungin, anidulafungin, caspofungin and micafungin against the fifth clade of *Candida (Candidozyma) auris*

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The aim of our study was to investigate the *in vivo* efficacy of rezafungin, anidulafungin, caspofungin and micafungin against *C. auris* isolates belonging to Iranian lineage. Three clinical isolates were evaluated (IFRC 4050, MRL40 and TMML616). MICs in RPMI-1640 were determined using the standard broth microdilution method (CLSI M27 ed4.). In the survival (ten mice/group) and fungal tissue burden experiments (nine mice/group), cyclophosphamide treated BALB/c male mice were infected intravenously (10^7 CFU/mouse, respectively). Treatment was initiated 24 hours post-infection with intraperitoneal dosing of 20 mg/kg of rezafungin (Rezzayo®) on days 1, 3 and 6 or once-daily dosing for 6 days with 3 mg/kg of caspofungin (Cancidas®), 5 mg/kg of micafungin (Mycamine®) or 5 mg/kg of anidulafungin (Eraxis®). After 21 days, survival rates were compared using the Kaplan-Meier log-rank 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 Periodic Acid Schiff was also performed. MIC ranges of rezafungin, anidulafungin, caspofungin, and micafungin were 0.06-0.25, ≤0.03-0.12, 0.12-0.5 and ≤0.03-0.12 mg/L, respectively. The four echinocandins improved the survival in mice infected with isolates MRL40 and IFRC 4050 (P-values were ≤0.0002 and 0.0006, respectively). In contrast, against mice infected with isolate TML617, only rezafungin improved the survival (P = 0.0049). All four echinocandins induced more than 5 and 4 logs mean CFU/gram decreases in the kidneys (P<0.001) and hearts (P<0.001 for all echinocandins), respectively in mice infected with isolate MRL40. In mice infected with isolate TML617 and IFRC 4050 the four echinocandins produced <3-log CFU mean fungal kidney and heart burden decreases some of which were not statistically significant. Fungal growth was poorly inhibited by echinocandins in the brain. Histopathology showed large aggregates of blastoconidia, budding yeast cells and pseudohyphae in the hearts, kidneys and brains in control mice. In echinocandins treated mice fungal cells were always visible in cerebrum or cerebellum. *In vivo* efficacy of the four echinocandins against the fifth clade of *C. auris* was echinocandin- and isolate-specific. Rezafungin activity was comparable to or better than the three previously approved echinocandins.

Bacterial community of the grapevine in conventional and organic vineyards in Badacsony

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The objective of this study was to investigate the microbial diversity associated with wine grapes (Vitis vinifera L.) in two distinctly managed vineyards (conventional and organic) in Hungary. Sampling was carried out in the Badacsony wine region, involving two white grape varieties: Kéknyelű, a local cultivar characteristic of the region, and Pinot Gris, an internationally widespread variety, both grown under conventional and organic agricultural practices. Samples were collected in the spring from inflorescence and shoots/leaves of the grapevines, and in autumn, sampling of berries will follow. It was hypothesised that the abundance and composition of microbial communities would differ across plant tissues and cultivation types, based on the different vineyard management systems. In total, 192 microbes were isolated in May 2025, and these microbial strains were incubated under aerobic and anaerobic conditions using different culture media. A subset of the isolates, 96 (50%), were

successfully identified using Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight mass spectrometry (MALDI-TOF MS). The preliminary data, which focused on bacteria, showed that the most abundant genera identified were Bacillus (19.79%), Pseudomonas (16.67%), Lactococcus (12.50%), Pantoea (9.38%), and Weissella (5.21%). The results indicate that among the lactic acid bacteria, Lactococcus spp. predominantly occur on the inflorescence, whereas Weissella spp. are primarily associated with the shoots/leaves. Pseudomonas spp. are more abundant on the inflorescence, while Bacillus spp. are more frequently found on the shoots/leaves. Pantoea agglomerans is mainly detected on the shoots/leaves, although it can also be present on the inflorescence. Samples that yielded unreliable MALDI-TOF MS scores (in total 24 isolates out of 96, 25%) will be subjected to further taxonomic resolution using molecular identification techniques.

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Microbial community composition of oyster mushroom substrate: Impacts of nucleic acid isolation and sequencing platforms

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Oyster mushroom (Pleurotus ostreatus) is one of the most common edible mushrooms cultivated on an industrial scale. The microbial community composition of the mushroom substrate is interesting in itself, if we would like to understand microbial background of Pleurotus production. But it is also an important information if we would like to further utilise the byproduct, the spent mushroom substrate (SMS). In the past we monitored regularly the bacterial community composition of the mushroom substrate on the DNA level. The aim of the present study to compare the bacterial and fungal community composition (1) on DNA and RNA level and (2) with using different sequencing platforms. Oyster mushroom substrates samples were collected after first and second flush of fruiting body production. RNA and DNA were isolated using the RNA PowerSoil® Total RNA Isolation Kit supplemented with RNA PowerSoil® DNA Elution Accessory Kit. DNA was isolated additionally with DNeasy® PowerSoil® Pro Kit. Prokaryotic 16S rRNA gene was amplified by the Earth Microbiome primer pair, whereas fungal nuclear rRNA ITS2 region was amplified by the ITS3_KYO2 - ITS4 primer pair. The products were analysed by Next Generation Sequencing on the Illumina platform. The raw sequences were processed and analysed using the pipeline of the SEED2 software. One DNA sample was also analysed via Oxford Nanopore and MGI. Comparisons were carried out with the R software. The main finding was, that RNA and DNA based community composition are not substantially different, but there are slight variations. Detailed analysis and effect of sequencing platform will be presented.

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Coding-complete genome sequence determination of an infectious laryngotracheitis virus from tracheal mucosa sample shipped on FTA card

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Infectious laryngotracheitis (ILT) is a highly contagious disease of chicken caused by the Infectious laryngotracheitis virus (ILTV) also known as the Gallid herpesvirus 1. The disease characterized by

severe or mild respiratory distress, tracheitis and conjunctivitis accompanied by egg production loss. ILT causes great economic losses due to morbidity, moderate to severe mortality and reduction of egg production. ILTV belongs to the genus Iltovirus and subfamily Alphaherpesvirinae within the Orthoherpesviridae family. ILTV possess a linear 150-155 kb long double stranded DNA genome consisting of the unique long (UL) and unique short (US) sequences flanked by inverted repeats (IR) and terminal repeats (TR). The genome encodes 80 open reading frames (ORFs), 65 ORFs located in the UL, 9 in the US and 6 ORFs in the IR and TR regions. FTA cards are designed to stabilize and protect nucleic acids at room temperature, so they are generally used for sample and nucleic acid transportation without special conditions. Despite its simplicity transportation on FTA cards has several limitations, such as the fragmentation of nucleic acids. Organ samples from layer chickens were shipped to our lab on FTA cards from the Middle East for diagnostic purposes. The samples were positive with ILTV-specific real-time PCR, genome sequence data were needed for further typing of the virus. Viral nucleic acid was extracted from an approx. 1cm2 section of the FTA card using the QIAamp DNA Mini kit according to the manufacturer's instructions. DNA library was prepared using the Nextera XT DNA library kit following the manufacturer's instructions. The library was loaded into an i1 flow cell and iSeq 100 i1 Reagent v2 cartridge and run on an Illumina ISeq 100 instrument. Reads were analyzed and genome was assembled by reference mapping using the Geneious Prime software. Phylogenetic analysis of the sequence data was carried out using the Mega X software. Approx. 96600 reads were assembled to a reference sequence, and an approx. 152kb long genome sequence was obtained of the ILTV present in the diagnostic sample. The complete coding sequences were determined, and phylogenetic analysis revealed that the strain belongs to the wild-type strains, separated from the vaccine-derived and recombinant strains.

In the shadow of the solar flare: a study of antioxidant protection during low-intensity, short-duration proton irradiation in *Aspergillus nidulans*

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Microorganisms, including fungi, play a dual role in space travel: they present challenges but also offer opportunities. They can damage spacecraft systems, pose infection risks to astronauts, and contaminate water and crops in bioregenerative life support systems (BLSS). Additionally, they produce allergens and toxic metabolites, such as mycotoxins or harmful volatile compounds, complicating space missions. Preventing microbial contamination remains a significant challenge due to Earth-based pollution. However, microorganisms are integral components of the human microbiome, making their adaptation crucial for astronaut health and efficient BLSS operation.

Cosmic radiation, primarily composed of high-energy protons, poses substantial challenges for shielding. Short-duration solar particle events (SPEs) can be partially shielded against but still carry risks like radiation sickness, cancer, and degenerative diseases. Understanding how SPEs affect microorganisms is vital for ensuring mission success and crew safety. Research on Aspergillus nidulans exposed to a simulated SPE revealed transcriptional changes. Upregulated genes were linked to DNA repair, ribosome biogenesis and protein ubiquitination, while downregulated genes included those involved in glycolysis, respiration and antioxidant defense. This suggests that oxidative stress plays a critical role in fungal responses to proton radiation. The findings highlight the complexity of microbial adaptation to space environments and emphasize the need for further research into protecting astronauts and spacecraft from microbial risks. In conclusion, these insights contribute to understanding how microorganisms adapt to cosmic radiation, offering potential strategies to mitigate risks and ensure successful long-term space exploration.

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Temporal, spatial and age-related trends of cytomegalovirus seroprevalence in childbearing-aged women in Croatia

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Cytomegalovirus (CMV) remains an important public health concern among childbearing-aged and pregnant women due to the risk of congenital infections. This retrospective study analyzed seroepidemiological trends of CMV in Croatia in ten consecutive years. A total of 2838 women aged 16-45 years, tested between 2015 and 2024 as part of the routine TORCH profile, were included. CMV IgM and IgG antibodies were detected using a commercial ELISA. Samples with detected both IgG and IgM antibodies were additionally tested for IgG avidity to confirm or exclude primary infection. CMV IgG antibodies were detected in 2006 participants (70.6%). The annual variations in the seroprevalence (62.4 to 77.3%) showed borderline significance (p=0.062). A progressive increase in the IgG was observed, from 49.6% in the 16-20 year group to 77.5% in the 36-40 year group. Significant age differences between IgG-positive and IgG-negative individuals, as well as between IgM-positive and IgM-negative individuals, were observed. The median age was higher among IgG-seropositive participants compared to those who were IgG-seronegative (33 vs. 30 years). Conversely, IgM-positive individuals had a lower median age than IgM-negative individuals (31 vs 32 years). Notable regional differences in IgG seroprevalence were found: the City of Zagreb/Northern Croatia, 68.6%; Pannonian Croatia, 78.5%; Adriatic Croatia, 71.9%. However, differences between urban and suburban/rural settlements were not statistically significant. IgG seropositivity was higher among women with an unfavorable obstetric history compared to both non-pregnant women and those with normal pregnancies (85.3, 70.6, and 66.5%, respectively; p=0.002). Acute/recent CMV infections (IgM antibodies) were found in 278 participants (9.8%), with higher rates in younger women, decreasing from 13.6% to 6.7% across age groups. Logistic regression analysis identified age as a significant predictor of both CMV IgG and IgM seropositivity. Geographic region and obstetric history were significant predictors of IgG positivity, while settlement type was associated with IgM seropositivity.

Environmental transmission routes of emerging tobamoviruses and other stable plant pathogens

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Viruses with exceptionally stable virions are an increasing concern in plant health due to their ability to persist in the environment, enabling long-distance dispersal and contributing to localized outbreaks. Understanding the behavior of these resilient pathogens within agricultural systems—particularly their persistence in water, soil, compost and other organic fertilizers—is essential for improving epidemiological risk assessments. We have shown that contaminated irrigation water could serve as a transmission route for environmentally stable viruses and viroids, including potato virus Y, pepino mosaic virus, potato spindle tuber viroid, tomato brown rugose fruit virus (ToBRFV), and tomato mottle mosaic virus (ToMMV) [1, 2]. Preliminary studies have indicated that ToBRFV and ToMMV can infect tomato plants when they are grown in contaminated substrate, further underscoring the significance of environmental stability in the transmission of these pathogens.

Our ongoing research is now exploring the survival of stable viruses and viroids in compost and other organic fertilizers. These materials are increasingly used in sustainable and circular agriculture, yet their potential to act as reservoirs or vectors for plant pathogens—especially via root-mediated infection—remains insufficiently studied. Addressing these challenges across irrigation water, soil, and organic fertilizers requires integrated approaches that combine sensitive molecular diagnostics (e.g., real-time PCR), untargeted high-throughput sequencing (HTS), enhanced with virus concentration methods, and controlled exposure experiments designed to simulate realistic agricultural conditions. These experiments are crucial for evaluating the potential for virus transmission and assessing the associated risks posed by stable plant viruses and viroids present in irrigation water, soil and organic waste-based fertilizers under field-relevant scenarios. By characterizing the survival and infection pathways of environmentally stable plant pathogens in soil, organic fertilizers and irrigation systems, our study clarifies underexplored transmission routes that influence disease dynamics. This knowledge supports risk assessment and informs management strategies to safeguard plant health and productivity in sustainable agricultural systems.

1 Mehle et al. 2014 Appl. Environ. Microbiol 80, 1455–1462
2 Mehle et al. 2023 Front. Plant Sci 14, 1187920

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Comparative Analysis of Rhizosphere Bacterial and Fungal Communities in Three Mentha Species

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Mentha species are globally cultivated aromatic plants valued for their high essential oil content and are known for their antimicrobial activity. These essential oils are released into the environment through root exudation, volatilization, and decaying plant material, where they may selectively inhibit or stimulate specific microbial taxa in the soil, influencing soil bacterial and fungal composition. Despite their widespread cultivation, the rhizosphere microbiome of Mentha species remains largely unexplored. This study aimed to characterize and compare the bacterial and fungal communities in the rhizosphere of Mentha × villosa (B10) and its parental species, M. spicata (B17), and M. suaveolens (J17) using metagenomic methods. Rhizosphere soil samples, with three biological replicates per species, were collected, and amplicon sequencing (16S rRNA for bacteria, ITS for fungi) was performed on the Illumina HiSeq platform. Data analysis was conducted using FROGS (a metagenomic pipeline) and R, focusing on taxonomic identification, alpha and beta diversity, and differential abundance analysis (DESeq2, LEfSe). The dominant bacterial phyla identified were Actinomycetota, Pseudomonadota, and Acidobacteriota, with mean relative abundances of 29.67%, 23.23%, and 13.64%, respectively. Among fungi, the most prevalent phyla were Ascomycota, Mortierellomycota, and Basidiomycota, exhibiting average relative abundances of 86.19%, 7.40%, and 2.32%, respectively. Interestingly the fungal community analyses revealed higher variability between replicates and between samples than bacterial communities, with differences more pronounced in fungal diversity measures. Alpha diversity indices and beta diversity analyses indicated differences among Mentha species, with B17 generally exhibiting lower diversity than B10 and J17. Differential abundance analyses showed limited but notable differences between Mentha species, with B10 and J17 being more similar, while B17 differed more significantly, particularly in fungal community profiles. Although it remains challenging to determine whether these differences originate from the plants themselves, essential oil effects, or soil-derived factors including

mycorrhizal associations or other endophytes, our results indicate that Mentha species can influence their rhizosphere microbiota composition, with potential environmental implications.
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Recent Advances in Oxygenic Denitrification: Potential Mechanisms and Environmental Relavance

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Although the microbial nitrogen cycle has been studied for over a century and a half, novel processes and responsible microorganisms continue to be discovered. One such process is oxygenic denitrification, initially identified in the anaerobic methanotrophic NC10 bacterium Candidatus Methylomirabilis oxyfera. This organism paradoxically requires oxygen for methane oxidation, which it generates internally via nitric oxide (NO) dismutation. Unlike conventional denitrification, where NO is sequentially reduced to nitrous oxide (N_2O) and then to nitrogen gas (N_2), oxygenic denitrification involves the disproportionation of NO into N_2 and O_2—a process termed NO dismutation (NOD). This pathway is both mechanistically intriguing and environmentally significant, as it bypasses the production of N_2O, a potent greenhouse gas, while simultaneously generating molecular oxygen. Targeted screening of nod genes encoding NO dismutase, along with environmental metagenomic analyses, has revealed a highly diverse and widely distributed nod gene pool across various ecosystems, including biofilms, aquifers, ditches, lakes, cold springs, agricultural soils, and wastewater treatment plants. These environmental nod sequences form distinct phylogenetic clades spanning multiple bacterial phyla; however, the taxonomic identities of most oxygenic denitrifiers remain elusive. Beyond methane oxidation by NC10 bacteria, recent studies suggest that oxygenic denitrification may also be involved in the degradation of aromatic hydrocarbons and ammonia oxidation. Cumulative molecular evidence indicates that oxygenic denitrification could represent a widespread and versatile microbial metabolism. In this context, we discuss the current understanding of the distribution, community composition, metabolic potential, and ecological significance of oxygenic denitrifiers, particularly in relation to nitrogen cycling and mitigation of N_2O emissions.
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Molecular epidemiology of the HIV-1 epidemic in Hungary until 2024

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HIV-1 causes a lifelong, incurable infection; thus, monitoring its transmission patterns is essential for improving prevention strategies. Based on routine drug resistance genotyping data collected since 2008, we present the most extensive molecular epidemiological analysis of the HIV-1 epidemic in Hungary to date. In total, we analyzed 1120 partial pol sequences from Hungary up until 2024, along with 2199 unique international sequences. We performed subtyping, drug resistance analysis, maximum likelihood and Bayesian phylogenetic inference, as well as distance-based and

phylogenetic clustering and assortativity analyses to identify and characterize domestic HIV-1 transmission within Hungary and transmission links across national borders. From the Hungarian sequences, we identified 85 sequence clusters (with sizes ranging from 3 to 96), comprising 66.4% of all sequences. Members of 17 larger clusters (10+ sequences) tended to be younger, more likely to be men who have sex with men (MSM), and had higher CD4 counts and viral loads than patients not assigned to large clusters. 39 of the 85 clusters showed activity in 2023–2024, but only five expanded by at least five sequences. Assortativity analyses on monophyletic pairs showed that the MSM and heterosexual (HET) risk groups were mostly, but not entirely, separated in Hungary. The prevalence of non-B subtypes increased significantly over the study period, and the presence of non-B subtypes was associated with female sex and the HET risk group. When incorporating international sequences, we identified 133 clusters containing both Hungarian and international sequences, as well as 56 closely related monophyletic pairs. These and additional Bayesian phylogeographic analyses revealed dozens of independent viral introduction events leading to domestic transmission. The countries with the most evidence of direct epidemiological linkage with B subtype sequences were Germany, Poland, the UK, Italy, and Croatia and for non-B subtypes Romania (subtype F), Russia (subtype A), Spain (CRF02_AG) and Thailand (CRF01_AE). The HIV-1 epidemic of Hungary is sustained by both domestic transmission and repeated viral introductions from other European countries, the latter driving also an increasing genetic diversity. Large active transmission clusters hint at the role of short-lived highly active sexual (MSM) networks and our findings indicate a significant separation between MSM and HET risk groups.