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A B S T R A C T S

in alphabetical order of first authors on pages 2-112

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Mapping and Characterization of Arsenic-Oxidizing Rhizosphere Bacteria in Hungarian Rice-Growing Areas

Renáta Ábrahám¹, Erzsébet Baka¹, Máté Tóth¹, Ines Amara¹, Ákos Suhajda¹, Milán Farkas¹, Tímea Szalóki², Mihály Jancsó², András Táncsics¹

¹ Department of Molecular Ecology, University of Agriculture and Life Sciences, Gödöllő, Hungary

² Research Center for Irrigation and Water Management, University of Agriculture and Life Sciences, Szarvas, Hungary

Arsenic is a widespread soil and groundwater contaminant, with toxicity strongly dependent on oxidation state. As(V), predominant in aerated soils, is less toxic than As(III), which dominates under the flooded, anaerobic conditions typical of rice paddies. Since rice is a global staple food, arsenic mobilization and uptake in paddy soils pose a direct food safety risk. Soil microbes play a key role in arsenic biogeochemistry. Arsenite-oxidizing bacteria carrying the *aoxB* (*aioA*) gene convert As(III) to the less mobile As(V), potentially reducing arsenic mobility. Mapping these bacteria is essential for understanding arsenic cycling in rice paddy soils, especially in naturally contaminated regions such as parts of the Hungarian Great Plain along the Körös River.

The aim of our study was to detect and characterize arsenite-oxidizing bacteria from the rhizosphere of a rice field in Békés County, Hungary, with an arsenic concentration of 8–9 mg/kg, by comparing direct isolation with arsenic enrichment. Rhizosphere soil samples were cultured under selective conditions directly from serial dilutions of 10⁻⁴–10⁻⁸, as well as from 10⁻²–10⁻⁶ dilutions following a two-week enrichment in modified minimal medium containing 1 mM arsenic. The isolates were identified using MALDI-TOF analysis and subsequently subjected to *aoxB*-specific PCR testing. In addition, the entire microbial community of the rhizosphere was investigated using 16S rRNA gene amplicon sequencing; the results of this analysis are currently being processed.

Based on the preliminary results, 42 direct isolates and 37 enriched isolates were obtained. The success rate of MALDI-TOF identification was higher among the enriched isolates (84%) compared with the direct isolates (47%). From a taxonomic perspective, direct isolation revealed a considerably more diverse bacterial community, including representatives of the phyla *Proteobacteria*, *Actinobacteria*, and *Bacillota*, with genera such as *Pseudomonas*, *Acidovorax*, *Microbacterium*, *Streptomyces*, and *Priestia*, as well as the species *Fictibacillus arsenicus*, previously isolated from an arsenic-contaminated habitat. In contrast, enrichment resulted in a narrower, predominantly *Gammaproteobacteria*-dominated community, including species belonging to the genera *Acinetobacter*, *Enterobacter*, *Klebsiella*, and *Leclercia*. The *aoxB*-specific PCR analysis of the isolates, as well as the analysis of rhizosphere community DNA based on 16S rRNA gene amplicon sequencing, is currently ongoing.

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Identification and integration of [D1,2] stwintrons in *Xylariaceae* sp. FL1272 fungal genome

Norbert Ág¹, Ruqayah Ayad Abbas^{1,2}, Michel Flipphi¹, Levente Karaffa¹, Erzsébet Fekete¹

¹ Department of Biochemical Engineering, Faculty of Science and Technology, University of Debrecen. ² Juhász-Nagy Pál Doctoral School of Biology and Environmental Sciences, University of Debrecen, Debrecen, Hungary

Introns were originally defined as non-coding RNA sequences that have to be precisely removed from the single-stranded primary transcript (pre-mRNA) before translation. In Eukaryota, spliceosomal introns are excised and neighboring exons are spliced together by the major U2 spliceosome. The assembling spliceosome recognises paired 5'- and 3'-splice sites at the opposite termini of the intron (called donor- and acceptor elements) within the pre-mRNA as well as an internal element containing the branchpoint adenosine that initiates the first of the two transesterification reactions of splicing. In fungi, there are quite a few genes that do not have interrupted orfs while ortholog genes in related species do. Introns thus seem selfish sequence elements that appear and disappear during evolution to randomly interrupt coding sequences. A vexing issue is how introns are continuously generated at previously unoccupied loci.

Continuous intronic sequences are not necessarily removed by one splicing reaction. We have studied the existence of spliceosomal twin introns or stwintrons in filamentous fungi (Ascomycota subphylum Pezizomycotina). Filamentous fungal U2 introns are usually small, often < 100 nt, and splice sites are paired by intron definition. Stwintrons consist of nested canonical U2 introns that consequently have to be removed by two canonical splicing reactions rather than by one. The configuration of two nested short U2 introns makes it possible to predict putative stwintrons from genome sequences. The most frequently occurring stwintron is [D1,2] where the donor of the external intron is interrupted by the internal intron between nts 5'-G₁ and U₂/C₂; The constituent U2 introns thus overlap 1 nt by their (opposite) terminal G.

Here, we present many hundreds of [D1,2] stwintrons in the *Xylariaceae* sp. strain FL1272 genome and a statistical analysis of the local sequence of their integration sites. We found intronic sequences that consist of a [D1,2] as the internal intron of a [D2,3] stwintron, and one "stwintron in a stwintron". We also found continuous intronic sequences consistent of two intact introns or of a stwintron and a U2 intron, without exon in between. We certified each stwintron candidate at the expression level (NCBI SRA SRX7708634), and by the manual prediction of intron-exon structures by comparative gene analyses in five other *Xylariaceae* sp., including FL1019. Stwintrons that are most sequence similar occur only in FL1272 (or only in FL1019), while stwintrons that are position conserved in FL1272 and FL1019 are likely generated before the divergence. Looking at the exonic borders of stwintrons, we detect a preference for integration between a T (5') and an A (3'), although all sixteen dinucleotide combinations are present in our sample. For phase one stwintrons, these are only UAU and UAC, leading to a striking overrepresentation of disrupted Tyr codons.

Whole-genome sequencing of bacterial strains derived from button mushroom cultivation

Henrietta Allaga^{1*}, Gábor Pál Balta¹, Rita Büchner¹, András Varga¹, Csaba Csutorás², Judit Bajzát², Sándor Kocsubé¹, László Kredics¹, Csaba Vágvölgyi¹, Tamás Papp¹

¹ University of Szeged, Department of Biotechnology and Microbiology, Szeged (Hungary)

² Új Champignons Ltd., Budapest (Hungary)

*Corresponding author: henrietta.allaga@gmail.com

The quality of compost and casing materials are essential prerequisites for efficient mushroom cultivation. Both substrates play a crucial role in fruit body development and contribute substantially to maintaining appropriate moisture levels. The microbial composition of the casing layer represents an important factor influencing its suitability for champignon production. The bacterial microbiota associated with spent *Agaricus* compost comprises a diverse range of genera, including *Bacillus*, *Alcaligenes*, *Pseudomonas*, and *Microbacterium*. Several species within the *Bacillus* and *Pseudomonas* genera have been reported to exert beneficial effects on mushroom cultivation. In particular, certain *Bacillus* and *Pseudomonas* species are considered important in mushroom production because of their antagonistic activity against pathogenic bacterial and fungal microorganisms, including *Trichoderma* spp. Through these interactions, such beneficial bacteria may contribute to the protection of mushroom cultures, support successful cultivation, and ultimately enhance both mushroom yield and quality under commercial growing-house conditions.

In our study, *Pseudomonas* strains were isolated and identified from casing layer samples collected during brown and white champignon cultivation. Additionally, we examined *Bacillus* strains isolated from spent mushroom compost. Strains showing promising results in ecophysiological and enzyme activity assays, fermentation condition optimization, chemical tolerance tests, and champignon cultivation experiments were selected for whole-genome sequencing. This approach enables a more detailed characterization of strains with favorable properties. As the number of chemicals permitted for use in mushroom cultivation continues to decline, beneficial *Pseudomonas* and *Bacillus* strains may provide a natural and environmentally friendly alternative for optimizing cultivation practices. Their application could reduce the dependence on chemical treatments and contribute to more sustainable mushroom production.

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Effects of PGP bacteria on tomato performance and associated microbiomes under contrasting irrigation regimes

Ines Amara¹, Milán Farkas¹, Francesca Mapelli², Lorenzo Vergani², Márton Pápai¹, Neveen Majdi Almalkawi¹, Sándor Takács³, Hussein Daood³, Mátyás Cserháti¹, András Tancsics¹, Balázs Kriszt⁴

¹ Department of Molecular Ecology, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

² Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Milan, Italy

³ Food Analysis Laboratories-Institute of Horticultural Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

⁴ Department of Environmental Safety, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary.

As water availability becomes increasingly unpredictable, microbial biostimulants such as plant growth-promoting (PGP) bacteria may help maintain crop productivity under water limitation. In summer 2025, we conducted a field trial evaluating two PGP strains, *Pseudomonas* sp. FSP16 and *Stenotrophomonas* sp. FSP12, in tomato under full irrigation (100%) and rainfed conditions (0%). In addition to the classical control, we also used a so-called control+ treatment, in which seedlings were grown in a nutrient-rich substrate instead of simple peat before transplanting to the field. Plant phenology, yield, fruit nutritional quality, and rhizosphere and endophytic bacterial communities were assessed. Both strains promoted plant growth under both irrigation regimes, although their effects were generally smaller than those observed in control+. Under rainfed conditions, FSP16 and FSP12 increased yield by 35% and 24%, respectively, suggesting a potential role in mitigating drought-related yield losses. Fruit quality responses were more pronounced under full irrigation: FSP12 increased tocopherol content by 24% and total carotenoids by up to 13%, while FSP16 increased total polyphenols by up to 29%. Rhizosphere bacterial communities were shaped primarily by irrigation regime, with lower diversity observed under drought. Both strains successfully colonized the rhizosphere, and one month after inoculation, 16S rRNA gene V3–V4 sequences assigned to the inoculant FSP12 and FSP16 strains were detected at substantially higher levels in drought-stressed plants than in control plants. Inoculant-associated sequences were also detectable under irrigated conditions, although at considerably lower levels. Analysis of the endophytic bacterial communities is currently underway and will offer further insight into how PGP inoculation shapes plant-associated microbiomes. Overall, the results indicate that PGP inoculation can enhance tomato performance under limited water availability, with strain-specific effects on yield, fruit quality, and rhizosphere bacterial communities.

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Structure and degradation capacity of bacterial communities originating from a natural hydrocarbon seep under aerobic and microaerobic conditions

Emma Bajzák¹, Erzsébet Baka¹, Renáta Ábrahám¹, Balázs Kriszt², András Tánácsics¹

¹Department of Molecular Ecology, Hungarian University of Agriculture and Life Sciences, Gödöllő, 2100, Páter K. street 1., Hungary

²Department of Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, 2100, Páter K. street 1., Hungary

Petroleum hydrocarbon contamination of groundwater and surface environments poses a persistent challenge, and indigenous microbial communities are key drivers of natural attenuation. Oxygen availability strongly shapes the composition and hydrocarbon-degrading capacity of these communities, yet direct comparisons of aerobic and microaerobic enrichment cultures established from the same inoculum are scarce.

We collected a sample from a natural crude oil seep in Poland and established parallel enrichment cultures under aerobic (dissolved oxygen: 7–8 mg L⁻¹) and microaerobic (~0.5 mg L⁻¹) conditions, in six replicates each (PA1–PA6 and PMA1–PMA6, respectively). Cultures were maintained for five weeks with weekly subculturing; dissolved oxygen consumption was monitored and adjusted continuously throughout the incubation. After the fifth passage, residual total petroleum hydrocarbon (TPH C5–C40) and BTEX content were determined by GC-FID/GC-MS, and the bacterial community composition was characterized by full-length 16S rRNA gene, as well as V3–V4 and V4 region amplicon sequencing.

Residual TPH concentrations were markedly lower in the aerobic enrichments (663–3230 µg dm⁻³) than in the microaerobic ones (976–27,000 µg dm⁻³), indicating more efficient hydrocarbon degradation under fully aerobic conditions, while BTEX components remained close to or below the detection limit in both series.

All three sequencing approaches consistently showed a strong enrichment of the phylum *Pseudomonadota* (from ~45–60% in the original seep sample to 80–93% in the enrichment cultures), with genus-level profiles dominated by *Pseudoxanthomonas*, *Stenotrophomonas* and *Parvibaculum* in both aerobic and microaerobic enrichments. *Hydrogenophaga* was characteristic of the aerobic cultures, while *Extensimonas* became dominant almost exclusively under microaerobic conditions. Full-length 16S rRNA gene sequencing resolved several taxa (e.g. *Extensimonas*) to genus level that short V4 amplicons could only assign to family level (*Comamonadaceae*), highlighting the added taxonomic resolution of long-read approaches for interpreting hydrocarbon-degrading consortia.

Our results demonstrate that dissolved oxygen concentration is a key factor shaping both the extent of hydrocarbon degradation and the composition of the enriched bacterial consortia, and that combining chemical and multi-resolution molecular data provides a robust framework for characterizing oil-degrading microbial communities.

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Origin or Oxygen: What really shapes hydrocarbon-degrading microbial communities?

Erzsébet Baka¹, Renáta Ábrahám¹, Emma Bajzák¹, Balázs Kriszt², András Tánicsics¹

¹Department of Molecular Ecology, Hungarian University of Agriculture and Life Sciences, Gödöllő, 2100, Páter K. street 1., Hungary

²Department of Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, 2100, Páter K. street 1., Hungary

Hydrocarbon contamination of soil and groundwater remains a persistent environmental problem, and understanding how hydrocarbon-degrading microbial communities assemble under different environmental conditions is essential for predicting and enhancing natural attenuation. In this study, we investigated the relative contributions of environmental origin and oxygen availability to the assembly of hydrocarbon-degrading enrichment communities. Parallel enrichment cultures were established under aerobic (7–8 mg L⁻¹ dissolved oxygen) and microaerobic (~0.5 mg L⁻¹ dissolved oxygen) conditions using inocula from two contrasting petroleum-impacted environments: a hydrocarbon-contaminated field groundwater and formation water from a crude oil well. Community composition was characterized by 16S rRNA gene amplicon sequencing. Permutational multivariate analysis of variance (PERMANOVA) revealed that both environmental origin ($R^2 = 0.44$, $p = 0.002$) and oxygen availability ($R^2 = 0.26$, $p = 0.006$) significantly structured community composition, with origin explaining the larger share of variance. At the phylum level, *Actinomycetota* proliferated under aerobic conditions, with *Dietzia* emerging as a characteristic aerobic genus that was consistently suppressed under oxygen limitation. *Pseudomonas* maintained a strong presence across both oxygen regimes, whereas *Acinetobacter* became a dominant genus specifically under microaerobic conditions, highlighting its ecological role under oxygen-limited settings. These results show that while environmental origin sets the baseline species pool available for enrichment, oxygen availability acts as a reproducible ecological filter that selects for functionally similar consortia regardless of the source environment. Our findings underline the importance of considering both historical (source-related) and environmental (redox-related) factors when predicting the composition and degradation potential of hydrocarbon-impacted microbial communities.

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Comparison of multiplex PCR-predicted resistance markers and phenotypic susceptibility testing

Bence Balázs¹, Bálint Farkas¹, Zoltán Tóth¹, László Majoros¹

¹ Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

The outcome of systemic and severe organ-specific infections—such as bloodstream, joint, or lower respiratory tract infections—is fundamentally determined by the rapid initiation of effective antimicrobial therapy. Although routine microbiological diagnostics have relied on culture-based methods on solid media for decades, the turnaround time of several days poses a significant limitation in acute patient care. In contrast, modern nucleic acid amplification-based assays can directly detect potential pathogens from primary clinical specimens with minimal preparation in approximately one hour, while also predicting their antimicrobial resistance profiles to a certain degree.

The primary advantage of closed-system multiplex platforms lies in their exceptional analytical sensitivity: they reliably identify fastidious or low-abundance organisms, while simultaneously detecting critical resistance genes (e.g., beta-lactamases, carbapenemases, glycopeptide-, and methicillin-resistance markers) to guide early therapeutic decisions. However, the fixed spectrum of predefined panels remains an inherent limitation, as targets outside the assay range remain undetected. Furthermore, high analytical sensitivity may blur the distinction between colonization and active infection.

In our retrospective study, we compared multiplex PCR results of clinical specimens (positive blood cultures, lower respiratory tract secretions, and synovial fluids) processed between 2022 and 2026 with the phenotypic susceptibility profiles (antibiograms) of the corresponding isolates. Particular attention was paid to the presence of ESBL (*bla*CTX-M), carbapenemase (*bla*KPC, *bla*NDM, *bla*OXA-48, *bla*VIM), and other resistance markers, as well as the rate of phenotypic resistance expression.

Molecular resistance prediction provides invaluable support for the timely de-escalation or escalation of empirical therapy, demonstrating excellent positive predictive value for the targeted genes. Nonetheless, due to non-target or non-genotypic resistance mechanisms, conventional phenotypic susceptibility testing remains indispensable for the safe guidance of definitive antibiotic therapy. The integrated, complementary use of both diagnostic modalities provides the most reliable support for clinical decision-making.

Exploiting the potential of an archival *Trichoderma* collection for future practical application

Dóra Balázs¹, Henriett Hunkár^{1,2}, Gergő Terna^{1,2}, Fanni Kovács^{1,2}, Ákos Rozsnyói^{1,2}, Chetna Tyagi¹, András Szekeres¹, Mónika Varga¹, Zoltán Naár³, Gyula Oros⁴, Csaba Vágvolgyi¹, Tamás Papp¹, Tamás Marik¹, László Kredics¹

¹Department of Biotechnology and Microbiology, University of Szeged, Szeged, Hungary

²Doctoral School of Biology, University of Szeged, Szeged, Hungary

³Department of Viticulture and Oenology, University of Tokaj, Sárospatak, Hungary

⁴Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary

The frequent extreme weather conditions and newly spreading plant pathogenic microorganisms urge the search for new, sustainable and safe solutions in the agriculture. Due to their favourable properties, small peptides produced by filamentous fungal *Trichoderma* species may provide new avenues in biological plant protection. Peptaibiotic compounds are short, biologically active peptides with characteristic properties, resistant structure and strong biological effects, which make them candidates for practical application.

In our work, we process an archival collection of *Trichoderma* strains established in the 1990s and isolated from different areas of Hungary, determine their phylogenetic position and produced peptaibiome, and select strains for potential biological plant protection purposes.

The revival of isolates in very poor condition was extremely successful, with more than 77% of the investigated samples viable, which have been determined as species such as *Trichoderma harzianum*, *T. virens*, *T. gamsii*, and *T. simmonsii*. A rapid peptide detection method was applied on the isolates, where extracts from the biomass and media were prepared using solvents and their biological activity was tested. The total peptaibiome of 15 strains producing bioactive extracts was determined by analytical mass spectrometry measurements, furthermore 9 strains were selected for large-scale peptaibiotic-production. The selected peptaibiotic mixtures were subjected to comprehensive investigations against various plant pathogenic microorganisms that cause significant damage in agriculture and were also involved in plant and ecotoxicological studies to establish their future safe practical application.

In addition to providing a comprehensive investigation of domestic *Trichoderma* strains of Hungary, our results also offer new opportunities for the development of modern biological plant protection methods.

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In vivo efficacy of antifungal agents against echinocandin-resistant *Candida auris* isolates

Dávid Balázs¹, Andrew Borman³, Gergely Udvarhelyi^{1,2}, Fruzsina Kovács¹, Andrea Németh-Harmath^{1,2}, Fruzsina Nagy¹, Zoltán Tóth¹, Renátó Kovács¹, László Majoros¹

¹ Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary

² Doctoral School of Pharmaceutical Sciences, University of Debrecen, Debrecen, Hungary

³ National Mycology Reference Laboratory, Bristol, UK

Candida auris is a multidrug-resistant fungal pathogen that was first isolated in 2009. A significant proportion of the isolated *C. auris* strains exhibit resistance to the most commonly used antifungal agents, including fluconazole and amphotericin B.

In our study, we investigated the *in vivo* efficacy of caspofungin, rezafungin, anidulafungin and posaconazole against various *Candida auris* isolates belonging to the South Asian clade in a neutropenic BALB/c mouse model. We used three isolates exhibiting varying degrees of resistance to echinocandins.

In both the lethality and fungal burden experiments, 10 mice were treated with the different drugs for 5 days. In the lethality experiments, observation continued until day 21, whilst in the fungal burden experiments, post-mortem examination was carried out on day 6.

In strain 208, 70 % of individuals treated with posaconazole survived, compared with 40 % of those treated with rezafungin. All individuals in the other groups died around the 5th day. In strain 28, posaconazole also proved to be the most effective antifungal agent, with a survival rate of 90 %. In the other groups, we observed a survival rate of close to 50 %. In contrast, for strain 20, 100 % of those treated with anidulafungin survived. For rezafungin and posaconazole, the survival rates were 90% respectively; for the other drug groups, the survival rate was never less than 50 %.

Strain 20 was used in the tissue persistence experiment. The organs examined were the brain, kidney and heart. In the case of the kidney, the number of viable fungal cells decreased significantly following posaconazole treatment, compared with both the day 1 and day 6 control groups. With regard to the heart, we observed a significant reduction compared with both daily control groups following treatment with all drugs; in contrast, in the brain, the number of fungal cells decreased significantly compared with the day 6 control group only following treatment with posaconazole and rezafungin.

Based on these data, posaconazole is highly effective in the treatment of echinocandin-resistant *Candida auris* infections.

Screening and characterization of probiotic microorganisms in the nematode *Caenorhabditis elegans*

János Barna^{1,2,3}, Sarolta Sipos¹, Zalán Árpási¹, Viktor Vázsony Vincze¹, Helga Szalontai³, Judit Kosztik³, Ildikó Bata-Vidács³, Tibor Vellai^{1,2}, István Nagy³, József Kukolya³

¹Department of Genetics, Eötvös Loránd University, Budapest, Hungary ²Genetics Research Group, HUN-REN-ELTE, Budapest, Hungary ³Food and Wine Research Institute, Eszterházy Károly Catholic University, Eger, Hungary

The gut microbiota comprises the diverse community of microorganisms inhabiting the intestinal tract. These microorganisms contribute to numerous physiological processes and influence host biology through complex mechanisms that remain only partially understood. Symbiotic bacteria are particularly important for the synthesis of certain vitamins and the regulation of immune functions. Alterations in the gut microbiome have also been associated with the development of certain cancers and neurodegenerative diseases. Moreover, age-related changes in gut microbiota composition may influence healthspan and lifespan.

Caenorhabditis elegans is a widely used model organism for studying aging and screening bacteria for potential probiotic properties. Its low maintenance costs, transparent body, short lifespan, and ease of experimental manipulation make it particularly suitable for large-scale screening.

The aim of our research is to screen and characterize bacterial strains with potential probiotic properties from a well-defined collection of several hundred *Lactobacillus* isolates of diverse origin. Using *C. elegans* as a model, we aim to identify bacterial strains capable of slowing aging, enhancing stress resistance, and ameliorating phenotypes associated with neurodegenerative diseases.

To achieve this, we are developing an optimized protocol for the rapid and efficient identification of bacterial strains with beneficial effects. Interestingly, we found that *C. elegans* fed with several strains belonging to the *Lactobacillaceae* family exhibited an extended lifespan compared with animals maintained on the standard laboratory food source, *Escherichia coli* OP50. However, animals fed exclusively with *Lactobacillaceae* strains also displayed larval arrest and reduced body size. Furthermore, food-choice assays revealed a preference for *E. coli* OP50 over *Lactobacillaceae* strains. Together, these observations suggest that the observed lifespan extension may result, at least in part, from the lower nutritional quality or reduced consumption of *Lactobacillaceae* strains rather than from a specific probiotic effect.

We are therefore currently optimizing our screening protocol by using mixed *E. coli*–*Lactobacillaceae* food sources to distinguish specific beneficial effects of bacterial strains from those caused by reduced food intake or dietary restriction. In parallel, we are investigating the mechanisms underlying the lifespan-extending effects of selected strains by monitoring the activity of reporter genes of pathways regulating longevity and cellular stress responses. These approaches may provide a robust *C. elegans*-based platform for identifying and characterizing bacterial strains with genuine probiotic potential.

Liver Abscess Mimicking Malignancy with Presence of *Olsenella uli* and *Actinomyces israelii*

Noémi Anikó Bartha¹, Mária Molnár¹, Olga Bárány¹, Márta Csire¹, Tibor Kovács², Attila Patócs¹

¹Department of Microbiology, Central Laboratory, National Institute of Oncology, Budapest, Hungary; ²Department of Abdominal Surgery, Cancer Surgery Center, National Institute of Oncology, Budapest, Hungary

Diagnosing liver abscesses can be particularly challenging when imaging studies reveal lesions that mimic hepatic malignancy. Recognition of anaerobic liver abscesses strongly depends on the quality of microbiological sampling and the proper execution of anaerobic culture techniques.

A 46-year-old man was evaluated for a painless palpable mass under the right costal margin. Ultrasonography, MRI, and CT scans all suggested primary liver malignancy with multiple pulmonary metastases. His medical history included chronic oral septic focus, brain abscess, and bilateral pneumonia, raising the possibility of hematogenous dissemination. The Gram-stained smear prepared from the aspirated material showed numerous leukocytes and Gram-positive rods, which early on suggested the presence of *Actinomyces*. Accordingly, special attention was paid to anaerobic culturing; the pathogens grew successfully within standard incubation period, and the culture confirmed the presence of *Olsenella uli* and *Actinomyces israelii*, indicating an anaerobic infection of oral origin. The patient improved following percutaneous drainage and the initiation of long-term antibiotic therapy targeted at the cultured pathogens.

This case highlights that rare anaerobic pathogens can produce liver lesions that closely mimic malignancy and are difficult to distinguish from true neoplastic disease on imaging. Early Gram-stain evaluation may provide crucial diagnostic guidance, while appropriate sampling and anaerobic culture enable accurate pathogen identification and initiation of targeted therapy.

Effects of microbial inoculants on reducing *Aspergillus flavus* infection and aflatoxin accumulation in a field trial on maize

Ildikó Bata-Vidács¹, Judit Kosztik¹, Mónika Varga², András Szekeres², József Kukolya¹

¹Centre of Research and Development, Eszterházy Károly Catholic University, H-3300 Eger, Hungary

²Department of Microbiology, Faculty of Science and Informatics, University of Szeged, H-6726 Szeged, Hungary

Abstract

Aspergillus flavus infection and the resulting aflatoxin contamination are important food and feed safety concerns in maize production. This field trial evaluated three *Aspergillus oryzae* strains (30, 34 and 40) and a *Trichoderma*-based inoculant, applied at the same concentration, for their potential to reduce aflatoxin accumulation. Culturable soil fungi were monitored at three time points during the growing season, while aflatoxin concentrations were determined from maize ear samples at the end of the trial.

All inoculant treatments resulted in lower aflatoxin concentrations than the infected untreated control. The available control measurements had a mean aflatoxin concentration of 1.84 mg/kg, whereas treatment values ranged from 0.70 to 1.25 mg/kg, corresponding to reductions of approximately 32–62%. The lowest aflatoxin concentrations were observed following treatment with *Trichoderma* and *A. oryzae* 34.

The soil mycological results showed marked treatment-specific changes over time. Total culturable *Aspergillus* abundance did not show a simple relationship with aflatoxin concentration, particularly because the applied *A. oryzae* strains may also have contributed to the *Aspergillus* counts. The different temporal patterns observed among the *A. oryzae* strains suggest differences in their persistence and effects on the culturable soil fungal community. These findings indicate that aflatoxin reduction cannot be explained solely by changes in the abundance of individual fungal groups and may involve more complex, treatment-specific interactions within the soil–plant microbial environment.

As aflatoxin determination was based on pooled samples for each treatment, the results should be regarded as preliminary evidence of biological effects rather than statistical confirmation. Nevertheless, the magnitude of the observed aflatoxin reduction, together with the temporal changes in the soil fungal community, identifies *Trichoderma* and *A. oryzae* 34 as promising candidates for further field evaluation using independent replicate-level aflatoxin measurements.

Whole-genome based characterization of pathogenic *Escherichia coli* strains isolated from Hungarian livestock

Andrea Béres¹, Linda Falgenhauer², Domonkos Sváb¹

1 Enteric bacteriology and alimentary zoonosis research group, HUN-REN Veterinary Medical Research Institute, Budapest, Hungary 2 Hessisches Landesamt für Gesundheit und Pflege, Außenstelle Dillenburg, Dillenburg, Germany

Numerous strains of pathogenic *Escherichia coli* are zoonotic pathogens enteric pathogens posing a significant health risk worldwide. Their remarkable genomic plasticity, made possible by mobile genetic elements, together with their wide repertoire of virulence and resistance determinants necessitates their monitoring and in-depth genomic characterisation.

In our study we determined the whole genome sequence of 34 *E. coli* strains isolated from various livestock from farms throughout Hungary using Nanopore sequencing. Gene content analysis revealed that the strains represented the following pathotypes: 8 Shiga-toxigenic (STEC, all of them originating from bovine), of which 4 were enterohemorrhagic (EHEC). Furthermore, there were 5 enteropathogenic strains (EPEC, 4 bovine, 1 originating from chicken), as well as 10 enterotoxigenic (ETEC, 7 bovine, 3 originating from chicken). Interestingly, one strain carried both the determinants of heat-labile enterotoxin and intimin, thus being EPEC/ETEC hybrid; and one carried the *stx* genes encoding Shiga-toxin as well as those encoding heat-stable enterotoxin, therefore representing an ETEC/STEC hybrid strain. We have identified resistance determinants conferring resistance against 9 antibiotic groups, including aminoglycoside, amphenicol, beta-lactam, folate pathway antagonist, fosfomycin, macrolide, polymixin, quinolone, tetracycline, with 24 strains being multidrug-resistant. *In silico* multi-locus sequence typing (MLST) showed that most of the strains represented ambiguous sequence types, with only 3 [2 from chicken/ 1 ETEC, 1 from rabbit, UPEC] strains representing ST10 and 2 [from bovine, not specific patotype] strains representing ST1146.

Our work underlines the diversity, the continuous genetic recombination and zoonotic potential of pathogenic *E. coli* in Hungarian livestock.

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Rootstock and the extent of water deficit shape the grapevine leaf mycobiome

Ramóna Biró-Kovács^{1,2}, József Geml^{1,3}, Ádám István Hegyi¹, Tibor Kiss¹, Dorina Veinémer³, Zsolt Zsófi³

¹Food and Wine Research Institute, Eszterházy Károly Catholic University, Eger, Hungary

²Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary

³Institute for Viticulture and Enology, Eszterházy Károly Catholic University, Eger, Hungary

Climate change, particularly increasing drought frequency and duration, represents a major challenge for sustainable viticulture. Rootstocks contribute to grapevine adaptation to water limitation, but their role in shaping drought-induced changes in the leaf-associated fungal microbiome remains poorly understood. This study investigated how short- and long-term water deficit and subsequent rewatering influence the leaf mycobiome of Furmint grafted onto different rootstocks using DNA metabarcoding.

A pot experiment was conducted with Furmint grafted onto Teleki 5C, Richter 110 and Paulsen 1103 rootstocks, which differ in drought tolerance. Plants were maintained at either 50% field capacity (water deficit) or 100% field capacity (well-watered control). Drought stress was imposed for 14 days (short-term) or 42 days (long-term), followed by rewatering. Leaves were sampled at 0, 24, 48 and 168 h after rewatering. Leaf-associated fungal communities were characterized by ITS2 rDNA metabarcoding. Sequence data were processed using DADA2, taxonomically assigned against the UNITE database using USEARCH and functionally annotated with FungiTraits. Community composition was evaluated using NMDS and PERMANOVA. Leaf fungal communities showed clear responses to water deficit and subsequent rewatering, with the magnitude and temporal dynamics of these changes varying among rootstocks and between short- and long-term drought treatments. Overall, prolonged water deficit resulted in more pronounced shifts in fungal community composition, while recovery after rewatering was characterized by dynamic changes over time. Rootstock also contributed to differences in community structure, suggesting that drought-induced changes in the leaf mycobiome are influenced by the rootstock–scion combination. In addition to changes in overall community composition, variation was observed in the richness of ecologically relevant functional groups, including plant pathogens and litter saprotrophs.

Our results demonstrate that drought-induced changes in the grapevine leaf mycobiome depend on drought duration, rootstock and recovery time. The stronger community responses following prolonged water deficit highlight the importance of considering rootstock-specific microbiome dynamics when evaluating grapevine resilience to increasingly frequent and persistent drought conditions.

The research was supported by the TKP-NKTA-16 research project.

Adaptation to Iron Limitation Enhances Resistance to Iron-Induced Stress in *Aspergillus fumigatus*

Vivien Bíró^{1,3}, Károly Antal², István Pócsi^{1,3}, Tamás Emri^{1,3},

¹ Department of Molecular Biotechnology and Microbiology, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, H-4032 Debrecen, Hungary

² Department of Zoology, Eszterházy Károly Catholic University, H-3300 Eger, Hungary

³ HUN-REN–UD Fungal Stress Biology Research Group, H-4032 Debrecen, Hungary

Iron limitation is a common stress that pathogenic microorganisms must overcome during infection. Interestingly, efficient iron efflux also contributes substantially to the virulence of human pathogenic bacteria, suggesting that the ability to cope with iron overload is likewise important for successful pathogenesis. Here, we investigated how the opportunistic human pathogenic fungus *Aspergillus fumigatus* survives exposure to excess iron. We hypothesized that adaptation to iron limitation, accompanied by the upregulation of iron acquisition processes, would increase the sensitivity of the fungus to FeSO₄-induced stress. Unexpectedly, cultures adapted to iron limitation exhibited enhanced growth compared with non-adapted reference cultures.

Transcriptomic analysis revealed that *cccA*, *fhpA*, and *gnoA* were consistently upregulated in response to FeSO₄ treatment across all experiments. In contrast, upregulation of genes involved in siderophore metabolism was characteristic specifically of cultures previously adapted to iron limitation. These findings suggest that CccA-mediated vacuolar iron sequestration, together with efficient protection against reactive nitrogen species mediated by the FhpA nitric oxide dioxygenase and GnoA S-nitrosoglutathione reductase, contributes to resistance to iron-induced stress. In addition, siderophore-mediated iron storage appears to play an important role in coping with excess iron in cultures adapted to iron limitation. The efficient production of siderophores by *A. fumigatus* may therefore explain the unexpected increased fitness of iron-limited cultures under iron stress.

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Characterization of the Soil Microbiome of the Tompapuszta Loess Grassland

Cserhádi Mátyás¹ Suhajda Ákos¹, Baka Erzsébet¹, Szegi Tamás², Guller Zsófia Eszter³, András István Csathó⁴, Micheli Erika²

¹Department of Molecular Ecology, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences (MATE), Páter Károly utca 1, 2100 Gödöllő, Hungary, ²Department of Soil, Institute of Environmental Science, Hungarian University of Agriculture and Life Sciences (MATE), Páter Károly utca 1, 2100, Gödöllő, Hungary, ³Department of Nature Conservation and Landscape Management, Institute for Wildlife Management and Nature Conservation, Hungarian University of Agriculture and Life Sciences, Páter Károly utca 1., H-2100 Gödöllő, Hungary, ⁴Independent Researcher, H-5830 Battonya, Hungary

Significant soil degradation processes can be observed across all major soil types in Hungary as a consequence of intensive arable farming. These processes affect not only soil structure, water-holding capacity, and nutrient-supplying potential, but are also associated with the depletion of soil microbiome diversity and richness, as well as disturbances in its functional properties. One of the objectives of our research is to determine the presumed baseline state of the microbiome richness and organic matter storage and accumulation capacity of the meadow chernozem soils of the Great Hungarian Plain prior to intensive agricultural disturbance. The Tompapusztai loess grassland represents one of the most unique core areas of nature conservation in Hungary. Its ancient and undisturbed status has been substantiated by botanical investigations conducted over the past two decades. This ancient status implies that, according to current knowledge, the area has remained undisturbed and has not been subjected to ploughing or other forms of agricultural cultivation for at least the past 1,000 years. Consequently, the original steppe grassland vegetation has been preserved on undisturbed chernozem soil, a feature considered unique in the areas east of the Tisza River. By now, the total area of the parcels placed under nature conservation protection, including the ancient grassland, exceeds 100 hectares. With the assistance of the national park authorities and botanical experts, we successfully conducted semi-invasive soil sampling across the area to a depth of 80 cm. Our objective is to characterize the soil physicochemical parameters and the soil microbiome using next-generation sequencing approaches in order to obtain as comprehensive a picture as possible of the microbial community inhabiting the site. In accordance with the genetic soil horizons, amplicon sequencing analyses are being performed at different depths using community DNA to characterize bacterial and fungal genera. In addition, multiplex PCR analyses are being conducted to assess the presence of genes associated with plant growth-promoting rhizobacteria (PGPR) traits, as well as mycotoxin-producing genes. The area also provides a unique opportunity to characterize, from a soil microbiome perspective, the processes of both spontaneous and assisted grassland restoration in adjacent areas managed by the national park. Thus, in addition to obtaining information from the undisturbed core area that has remained free from the impacts of conventional arable cultivation, we can compare the soil microbiomes of restored grassland areas that have remained undisturbed for 16, 8, and 3 years with those of the core area. This comparative approach will enable us to assess the effects of grassland restoration and habitat reconstruction on the soil microbiome. Our ultimate objective is to establish a comprehensive microbiome profile of the core area that can serve as an absolute reference point for comparative analyses of lowland meadow chernozem soils throughout the Carpathian Basin. In addition, we plan to investigate the rhizosphere microbiome associated with the root systems of the most abundant grass species characteristic of the grassland in 2027.

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Genetic analysis of HogA MAPK stress signalling towards the bZIP-type transcription factor AtfA in *Aspergillus flavus*

Viktória Csontos¹, Boglárka Pável¹, Dorina Nagy¹, Éva Leiter^{1,2}, István Pócsi^{1,2}

¹ Department of Biotechnology and Microbiology, Institute of Biotechnology, University of Debrecen, Debrecen, Hungary

² HUN-REN-DE Fungal Stress Biology Research Group, Debrecen, Hungary

Adaptation to environmental stress is essential for the survival and pathogenicity of *Aspergillus flavus*. MAP kinase (MAPK) signalling pathways play a central role in sensing environmental stressors and initiating responses to them, while the bZIP-type transcription factor AtfA regulates the expression of numerous stress-responsive genes in the Aspergilli. Although AtfA is considered functioning downstream of the HOG ("High Osmolarity Glycerol") MAPK pathway, the functional relationship between HOG MAPK signalling and AtfA in the aflatoxin-producing fungus *A. flavus* has not yet been fully elucidated.

The aim of the study was to genetically analyze the AflHogA MAPK-to-AflAtfA stress signaling by characterizing the stress-sensitivity phenotypes of MAPK and $\Delta AflatfA$ deletion mutants under various environmental stress conditions.

The $\Delta AflhogA$ and $\Delta AflatfA$ single mutant and the $\Delta AflhogA \Delta AflatfA$ double mutant strain were generated using Double-Joint PCR and protoplast-mediated transformation. The mutants were verified by PCR and restriction analysis and subsequently characterized under oxidative, osmotic, and cell wall stress conditions.

Deletion of the *AflatfA* gene resulted in increased sensitivity to oxidative and cell wall stress, whereas deletion of *AflhogA*, if it had any effect at all, was associated with only minor phenotypic changes. Notably, the phenotypes of the $\Delta AflhogA \Delta AflatfA$ double mutant closely resembled those of the $\Delta AflatfA$ strain. These results support the hypothesis that AflAtfA is a key transcriptional regulator of stress adaptation in *A. flavus*, however, its activation likely does not depend solely on AflHogA, as stress-dependent regulatory signals may also be transmitted towards AflAtfA via other MAPK pathways.

Our results provide new insights into the relationship between AflHogA MAPK signalling and the AflAtfA-dependent stress response in *A. flavus*. The observed phenotypes suggest that AflAtfA functions as a central integrator of stress signalling, implying that multiple MAPK pathways may contribute to its stress-dependent activation. We hope that ongoing studies involving additional *A. flavus* MAPK mutants will elucidate the signalling network regulating the AflAtfA-dependent stress response.

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Investigation of viruses and mixed infections in symptomatic pepper plants using high-throughput sequencing

Emese Demián¹, Réka Sáray¹, Asztéria Almási¹, Kata Pogácsás², Katalin Salánki¹

¹Department of Plant Pathology, Plant Protection Institute, Centre for Agricultural Research, Hungarian Research Network (HUN-REN), Budapest, Hungary; ²Horticultural Plant Genetics Group, Department of Plant Biotechnology, Institute of Genetics and Biotechnology, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary

Identifying viruses in symptomatic plants can be challenging when routine diagnostic methods fail to identify the cause of the symptoms. In this study, high-throughput sequencing (HTS) was used to investigate symptomatic pepper (*Capsicum annuum* L.) plants and to characterize their associated viral communities. Five symptomatic pepper plants collected from greenhouse production in Hungary between 2020 and 2025 were chosen for further analysis because the observed symptoms could not be fully explained. High-throughput RNA sequencing was performed, followed by bioinformatic analysis of the sequencing data. The detected viruses were confirmed by virus-specific RT-PCR, and the amplified viral regions were sequenced by Sanger sequencing for phylogenetic analysis.

HTS revealed a complex viral community, with six viruses detected in the five symptomatic plants: tomato spotted wilt virus (TSWV), pepper mild mottle virus (PMMoV), eggplant mottled dwarf virus (EMDV), tobacco vein clearing virus (TVCV), bell pepper alphaendornavirus (BPEV) and pepper cryptic virus 2 (PCV2). EMDV was detected in two pepper plants, and a near-complete consensus genome was reconstructed from the HTS data. This is the first confirmed detection of EMDV in pepper in Hungary. Phylogenetic analysis showed that the Hungarian EMDV sequences clustered with an Iranian isolate. TVCV-related sequences were detected in four plants and showed high similarity to endogenous pararetroviral elements in the *C. annuum* genome, suggesting a possible association with these elements. Persistent viruses, including BPEV and PCV2, were also detected and confirmed by RT-PCR.

Our results show that symptomatic pepper plants can be associated with complex viral communities, including mixed infections and virus-related sequences. HTS provided additional information on the viruses present in these plants and enabled the characterization of viruses detected in Hungarian pepper.

Hepatitis A virus outbreaks in Europe

Agnes Dencs

Department of Virological Reference Laboratories, National Center for Public Health and Pharmacy, Budapest, Hungary

The epidemiology of hepatitis A virus (HAV) infection has changed considerably in Europe over the past decades. Improvements in hygiene and socioeconomic conditions have led to reduced exposure during childhood, resulting in an increasing proportion of adults susceptible to infection. In countries with low and very low endemicity, a substantial proportion of infections are associated with travel to higher-risk areas or consumption of contaminated imported food. However, HAV continues to circulate within certain population groups, including people of low socioeconomic status, people who inject drugs, and men who have sex with men (MSM).

These populations remain in close contact with the wider population, which is largely susceptible to HAV infection, allowing the virus to periodically spill over into the broader community. Food handlers and workers in childcare settings can also contribute to the spread of the virus and the development of outbreaks of varying magnitude. Recent events in Europe illustrate this dynamic well. In addition to difficult-to-trace foodborne clusters, a major Europe-wide outbreak primarily affecting the MSM population occurred in 2016–2018, and the HAV strains responsible continue to re-emerge across the continent. Since 2023, a significant HAV outbreak has been ongoing in Central Europe, affecting Hungary, Slovakia, Czechia and Austria, and predominantly spreading among vulnerable populations living in poor sanitary conditions.

Declining natural immunity and the presence of susceptible populations, together with extensive international travel, food trade, and diverse transmission networks, continue to facilitate the spread of HAV. Hepatitis A therefore remains an ongoing public health challenge in Europe. Molecular surveillance and phylogenetic analysis of viral genomes are becoming increasingly important for detecting outbreaks, identifying transmission chains, and monitoring the spread of viral variants.

Characterisation of Nipah Virus Replication and Dissemination in Human Brain Organoids and 3D Spheroids

Dániel Déri¹, Jakub Hantabal², Levente Lakatos¹, Bereniké Novák¹, Gyula Zsidei¹, Miles W. Carroll², Zoltán Kis^{1,3,4}, Bernadett Pályi^{1,4}

¹ National Biosafety Laboratory, National Center for Public Health and Pharmacy, Budapest, Hungary ² Pandemic Sciences Institute and Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford, UK, ³ Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary ⁴ European Research Infrastructure on Highly Pathogenic Agents (ERINHA-AISBL), Brussels, Belgium

Nipah virus (NiV) is a zoonotic, highly pathogenic paramyxovirus of the genus *Henipavirus*, *Pteropus* fruit bats serve as its natural reservoirs. Currently there are no available specific antiviral therapy or vaccine, thus the inclusion in WHO R&D Blueprint. Both of its NiV-Malaysia (NiV-M) and NiV-Bangladesh (NiV-B) strains are associated with human outbreaks, but there are some differences in clinical presentation and epidemiology. Human symptoms ranges from asymptomatic/mild disease to severe respiratory illness and encephalitis, with case-fatality rates between 40-75%. NiV shows a broad cellular tropism primarily mediated by ephrin-B2 and ephrin-B3 receptors, endothelial and neuronal involvement causing systemic and central nervous system (CNS) infections. Despite its high epidemic potential and pathogenicity, there are many questions to be answered about the mechanisms of its neuropathogenesis. Beside 2D cell-cultures, 3D spheroid and human brain organoid models might provide a suitable, more relevant environment for investigating e.g. viral replication, tissue tropism and NiV-induced tissue damage. This study evaluated the susceptibility, replication kinetics, and viral spread of NiV-M and NiV-B in 3D Vero Nuvonis spheroids and human induced pluripotent stem cell (iPSC)-derived brain organoids. Furthermore, we assessed the efficacy of a candidate antiviral drug previously validated in 2D cell-culture and initiated spatial transcriptomic profiling to identify targeted cell populations and viral routes of spread. All laboratory work involving the live virus was conducted at the BSL-4 facility of National Biosafety Laboratory in Budapest, while spatial transcriptomics is being carried out in Oxford. Preliminary data confirm that both spheroids and human brain organoids support productive infection by both NiV-M and NiV-B strains. While 3D spheroids yielded lower overall viral loads compared to traditional 2D monolayer controls, their replication kinetics depended heavily on initial viral load – higher inocula closely mirrored 2D growth curves, whereas lower inocula resulted in a marked kinetic delay in viral replication. Conversely, human brain organoids demonstrated striking susceptibility, exhibiting robust, rapid viral expansion and high infectious progeny yield for both strains within days of infection. Antiviral treatment consistently suppressed viral replication in spheroids, though therapeutic efficacy in brain organoids was less pronounced, underscoring the need to optimize time-of-addition protocols for complex tissue models.

This study provides insights by establishing human iPSC-derived brain organoids as novel, permissive 3D tissue models for evaluating Nipah virus pathogenesis and antiviral efficacy.

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Transcriptomic profiling revealed global alterations in *Malassezia pachydermatis* isolates following short-term ketoconazole stress

Marianna Domán¹, Enikő Wehmann¹, László Makrai², Enikő Fehér³, Tibor Magyar¹

¹HUN-REN Veterinary Medical Research Institute, Budapest, Hungary

²Autovaccina Kft., Budapest, Hungary

³Department of Microbiology and Infectious Diseases, University of Veterinary Medicine, Budapest, Hungary

Malassezia pachydermatis is an opportunistic yeast causing otitis externa and dermatitis in dogs. Although reduced susceptibility to azole antifungals has increasingly been reported in *M. pachydermatis*, the molecular mechanisms underlying azole stress responses in this species remain poorly understood. In this study, we investigated the global transcriptional response of two *M. pachydermatis* isolates with different ketoconazole minimum inhibitory concentration (MIC) values following short-term ketoconazole exposure. Ketoconazole treatment resulted in slightly distinct transcriptional profiles in the two isolates. Both isolates exhibited upregulation of *ERG* genes, as well as genes encoding transport proteins, and those involved in protein folding and the oxidative stress response. Furthermore, in isolate 12693, several genes encoding ubiquitin ligases, RNA polymerase regulatory proteins, and proteins involved in lipid metabolism were upregulated in response of the treatment. Downregulation of genes responsible for ribosome biogenesis, amino acid biosynthesis, and nucleotide biosynthesis was observed in both isolates; however, the extent of downregulation was greater in isolate 12693. These transcriptomic alterations suggest a more extensive reprogramming of transcription and protein homeostasis in isolate 12693 following ketoconazole treatment compared to the response observed in isolate 12372. Interestingly, no detectable changes in the expression of genes encoding efflux pumps were observed in the treated isolates compared to the untreated controls. The rapid and extensive transcriptional response of isolate 12693 to ketoconazole stress may provide a potential explanation for its enhanced viability and higher ketoconazole MIC value compared with isolate 12372. Our study represents the first comprehensive, transcriptome-wide characterization of the response of *M. pachydermatis* to azole stress in the literature. These investigations are essential to determine whether transcriptional changes are associated with antifungal susceptibility, to elucidate the molecular mechanisms underlying azole adaptation and tolerance, and to identify potential targets for the development of novel antifungal agents.

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Emergence of giant viruses (*Mimiviridae*) in Hungarian sturgeon farms

Andor Doszpoly¹, Gergő Mitró¹, Márton Hoitsy^{2,3,4}, Mária Benkő¹, Balázs Harrach¹

¹ HUN-REN Veterinary Medical Research Institute, Budapest, Hungary

² University of Veterinary Medicine, Department of Exotic Animal-, Wildlife-, Fish- and Honeybee Medicine, Budapest, Hungary

³ Budapest Zoo, Budapest, Hungary.

⁴ Vet4Fish Kft. 2111 Szada, Hungary

In Hungary, sturgeon species are primarily raised in intensive recirculating aquaculture systems. The emergence of pathogens in such systems can lead to severe losses. Two years ago, just after the emergence of the Siberian sturgeon herpesvirus affecting sturgeon in Hungarian farms, another mass mortality event involving Russian sturgeon (*Acipenser gueldenstaedtii*) fry occurred at a different farm in Hungary. In the affected fry population, we observed the following clinical signs: abnormal swimming, petachial haemorrhages on the gills and in the body cavity, lethargy, and loss of appetite. During diagnostic testing of the animals, we found no pathogenic bacteria, nor were we able to detect the presence of herpesvirus. Through further molecular testing, we were able to confirm the presence of another pathogen, “Acipenser Iridovirus-European” (AcIV-E). This virus, which has not yet been officially classified taxonomically, has been detected in several European countries since 2016 (e.g., Italy, Poland, France). The virus causes significant mortality in sturgeon farms, primarily among juvenile fish (as high as 90–100%). AcIV-E is capable of infecting numerous sturgeon species and has been detected in beluga sturgeon (*Huso huso*), Siberian sturgeon (*Acipenser baerii*), Russian sturgeon, starry sturgeon (*A. stellatus*), and Adriatic sturgeon (*A. naccarii*). Similar (but not identical) viruses were first described in North America in the 1990s; based on partial genome sequences. Both of these viruses are most closely related to the giant viruses (*Mimiviridae*) described in amoebae.

In a retrospective study, we demonstrated that AcIV-E appeared in Hungary at the same time as the herpesvirus infection in 2023. The viruses likely entered the country from Poland through a restocking of the breeding stock. Since then, we have been able to detect the presence of AcIV-E—similarly to that of the herpesvirus—at least in three domestic farms every year. The isolation of the virus was successful and we could sequence the coding-complete genome. We have submitted a proposal to ICTV to establish a new species and a new genus for this virus in the family *Mimiviridae*.

It is important to emphasise that currently, neither a cure nor an effective commercially available vaccine is available. The focus must therefore be on prevention and compliance with animal health protocols.

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Environmental conditions during conidiogenesis modulate *Aspergillus fumigatus* virulence

Gréta Dudás¹, István Pócsi^{1,2}, Tamás Emri^{1,2}

¹ Department of Molecular Biotechnology and Microbiology, University of Debrecen, Hungary, 2 HUN-REN–UD Fungal Stress Biology Research Group

Several studies have demonstrated that the stress tolerance of fungal conidia can be influenced by the conditions under which they are produced. Such alterations in conidial stress tolerance may also affect fungal virulence in pathogenic species. To investigate this hypothesis, we assessed the *in vivo* virulence of *Aspergillus fumigatus* Af293 conidia produced under different culture conditions using the *Galleria mellonella* model of invasive aspergillosis.

Iron limitation induced by the iron chelator deferiprone decreased conidial virulence, whereas the presence of nitrate during conidiogenesis increased virulence. In contrast, the culturing temperature (24 °C vs. 37 °C) and the presence of sodium menadione bisulfite as an oxidative stress-inducing agent had no significant effect on virulence.

Based on these findings, we hypothesize that (1) conidial virulence is strongly influenced by their iron content and (2) increased tolerance to nitrosative stress induced by nitrate during conidiogenesis may contribute to the enhanced virulence of conidia. These results further highlight the importance of conidial origin when comparing outcomes of *in vivo* virulence assays, as differences in the conditions under which conidia are produced may represent an important source of variability.

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Physiological requirements and co-culturing of microorganisms isolated from an estrogen enrichment medium with the potential to degrade naturally occurring hormones

Emília Laura Dzsudzsák^{1,3}, Dávid Várkonyi², Balázs Göbölös¹, Péter Harkai¹, Julianna Kobolák², Balázs Kriszt¹, Edit Kaszab¹

¹ Department of Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

² Department of Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

³ Doctoral School of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

Estrogen hormones, particularly estrone (E1), 17 β -estradiol (E2), and estriol (E3), constitute a significant group of natural endocrine disruptors released into the environment. Microorganisms may play a key role in their biological removal; therefore, the study of bacteria capable of degrading hormones and their community interactions is an important area of research. The aim of this study was to investigate the individual and co-culture behaviour of potentially hormone-degrading bacteria isolated from an enrichment culture system with estrogens as solo carbon sources. Particular emphasis was placed on the role of culture conditions and interactions among microorganisms.

During the experiments, a total of eight bacterial strains, previously isolated from estrogen-enriched media, were selected. By testing the strains in individual cultures, we determined the pH, temperature, and aeration conditions that were optimal for their growth. Based on the optimized parameters, we then examined possible interactions between the strains using a cross-streak test. This method allowed us the preliminary assessment whether inhibitory or potentially symbiotic interactions could be observed between the individual isolates.

Based on the analysis of individual cultures, the selected strains exhibited different environmental requirements and growth optima. According to the results of the cross-streaking test, none of the tested bacterial strains showed any detectable growth-inhibition or antagonistic effects against the other isolates. Based on these results, there were no interactions among the tested strains that would unequivocally preclude co-cultivation; thus, it is possible to include the isolates in further combined co-culture experiments. The current results provide a solid foundation for the development of microbial co-cultures in which individual bacterial strains can potentially complement each other's metabolic activity and thereby contribute to a more efficient biological degradation of estrogen hormones.

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Sublineage-specific polymorphism modulates HPV11 E7 function: implications for viral pathogenesis

Zsolt Barnabás Éles^{1,2,3}, Leila Rahmani¹, Eszter Gyöngyösi^{1,4}, György Veress^{1,4}, József Kónya^{1,4},
Anita Szalmás^{1,4}

1. Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
2. Division of Clinical Laboratory Sciences, Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
3. Lendület “Momentum” Hemostasis and Stroke Research Group of the Hungarian Academy of Sciences, University of Debrecen, Debrecen, Hungary
4. Medical Microbiology, Clinical Center, University of Debrecen, Debrecen, Hungary

The human papillomavirus (HPV) E7 oncoprotein is essential for viral replication and pathogenesis. Although highly conserved within genotypes, naturally occurring E7 variants can exhibit distinct functional properties that influence viral persistence and host cellular responses. We functionally characterized the prevalent HPV11 A2 sublineage, which harbors a characteristic amino acid substitution (A45S) in the E7 protein.

Comparative analyses in transfected primary keratinocytes and HPV-negative cancer cells revealed that the A45S substitution enhanced HPV11 E7 binding to key host targets, specifically PTPN14 and pRb family proteins. This heightened affinity directly increased the rate of target protein degradation. Furthermore, the S45 variant displayed an enhanced capacity to drive E2F-mediated transcription. In a luciferase reporter assay, S45 HPV11 E7 transactivated the Ad E2 promoter to a level similar to HPV16 E7 and upregulated E2F-controlled genes, specifically increasing expression of key factors in the homologous recombination pathway for DNA double-strand break repair. Furthermore, S45 HPV11 E7-expressing primary keratinocytes displayed enhanced cell viability compared to A 45 HPV11 E7- and HPV16 E7-expressing cells. These findings indicate that modulation of DNA damage response pathways, which HPVs commonly exploit to support viral replication and persistence, may play a key role in the increased cell viability associated with the A45S substitution.

Together, these data demonstrate that the single A45S substitution confers a high-risk-like molecular phenotype on the low-risk HPV11 E7 protein. As the first report of a functionally consequential natural sequence variation in HPV11 E7, these findings offer mechanistic insights into viral pathogenesis and highlight potential targets for managing persistent clinical manifestations, including recurrent respiratory papillomatosis.

Taxonomic diversity of microbial communities in the permafrost active layer across elevation, depth, and time at Ojos del Salado (Atacama Desert, Chile)

Viktória Faragó^{1,2}, Andrea K. Borsodi¹, Balázs Nagy^{3,4}

¹ Department of Microbiology, Eötvös Loránd University, Budapest, Hungary ² Doctoral School of Environmental Sciences, Eötvös Loránd University, Budapest, Hungary ³ Department of Physical Geography, Eötvös Loránd University, Budapest, Hungary ⁴ A Földgömb az Expedíciós Kutatásért Alapítvány, Budapest, Hungary

The world's highest volcano, Ojos del Salado (6,893 m a.s.l.), is located in the harsh environment of the Atacama Desert (Chile). The area is characterized by extreme aridity, cold temperatures, high UV radiation, and a negative water balance. Polyextremophilic microbial communities inhabiting this high-altitude permafrost serve as an ideal model for exploring the limits of life on Earth and assessing microbial climate sensitivity. In this study, 16S rRNA gene amplicon sequencing was performed to characterize microbial taxonomic and alpha diversity in the permafrost active layer. To evaluate the potential influence of elevation, depth, and time on microbial community structure, samples were taken from three elevations (4,550 m, 5,260 m, and 5,830 m), three soil depths (10 cm, 35 cm, and 60 cm), and two sampling years (2023, 2025) on Ojos del Salado. A diverse bacterial community was observed (Shannon diversity: 4.77-6.72), dominated by Actinomycetota (41.75% relative abundance). Families *Nocardioidaceae* (10.33%) and *Chitinophagaceae* (7.40%) were highly abundant. By contrast, archaeal communities were characterized by low diversity and were dominated almost exclusively by the *Nitrososphaeraceae* family. Variations across the whole community were significantly driven by elevation and sampling year, whereas sampling depth had no significant effect. Our findings provide insights into microbial diversity shifts within the high-altitude permafrost active layer, contributing to a better understanding of Andean permafrost ecosystems exposed to the impacts of climate change.

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Antifungal susceptibility testing of filamentous fungi isolates from clinical samples

Bálint Farkas¹, László Majoros¹, Dávid Balázsi¹, Renátó Kovács¹, Katalin Kristóf², Ágnes Sonnevend³, Eszter Vad⁴, Judit Éva Szabó⁵, Katalin Papp⁶, Ilona Csányiné Dóczy⁷, Tamás Mlinkó⁸, Zoltán Tóth¹

¹ Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary 2 Institute of Laboratory Medicine, Semmelweis University, Budapest, Hungary 3 Department of Medical Microbiology, University of Pécs Medical School, Pécs, Hungary 4 South Pest Central Hospital, National Institute of Hematology and Infectious Diseases, Saint Ladislaus Campus, Core Microbiology Laboratory, Budapest, Hungary. 5 Department of Medical Microbiology, Petz Aladár University Teaching Hospital, Győr, Hungary 6 Microbiology Laboratory, Szabolcs-Szatmár-Bereg County Teaching Hospital – András Jósza Member Hospital, Nyíregyháza, Hungary 7 Department of Medical Microbiology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary 8 Microbiology Laboratory, National Korányi Institute for Pulmonology, Budapest, Hungary

Invasive fungal infections are considered to be a great burden on human health. The high mortality and morbidity are serious problems on their own, but the multiple comorbidities of many patients make managing these infections more difficult. This is exacerbated by the rising resistance rates and changing epidemiology globally. The data available is scarce regarding the aetiology and resistance rates of invasive mycosis in Hungary. To address this knowledge gap, we initiated a surveillance project, collecting fungal isolates of potentially invasive fungal infections from several diagnostic centres of the country, analysing species distribution and susceptibility data.

Between 2025 January and 2026 July, we collected 157 isolates of filamentous fungi considered clinically significant by the participating clinical microbiology laboratories, mainly but not exclusively from lower respiratory tract. Preliminary identification was performed with MALDI-TOF MS system and conventional methods. Antifungal susceptibility testing was performed using CLSI M38 ed3. microdilution method regarding filamentous fungi against Amphotericin B, Voriconazole, Posaconazole, Isavuconazole, Micafungin, Anidulafungin, Caspofungin and Rezafungin. Minimal Inhibitory Concentrations and Minimum Effective Concentrations were interpreted according to CLSI M38M51Sed3 and CLSI M57ed4.

Out of the 156 isolates 140 were found to be *Aspergillus* sp., 11 *Fusarium* sp., and 5 of the order Mucorales. *Aspergillus fumigatus* complex was the most common with 65 isolates followed by 49 *Aspergillus flavus-oryzae* complex, 10 *Aspergillus niger* complex, 9 *Aspergillus terreus* complex, and 7 *Aspergillus nidulans* complex. Based on phenotypic susceptibility data, only one isolate was non-susceptible to voriconazole, posaconazole and isavuconazole. Amphotericin B non-wild type isolates were not found. Rezafungin showed slightly weaker activity than anidulafungin and micafungin, and better than that of caspofungin, yet no caspofungin non-WT isolates were found. Confirmatory and molecular testing of the non-WT isolate is ongoing. The susceptibility profiles of non-*Aspergillus* moulds were in accordance with the literature.

Overall, the species distribution of *Aspergillus* species is in line with other studies, yet the high percentage of *A. flavus-oryzae* complex isolates was somewhat surprising. Antifungal non-susceptibility among filamentous fungi collected during the study period was extremely rare, suggesting that antifungal resistance among moulds is uncommon in Hungary in the clinical setting.

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The in vitro activity of antifungal agents against planktonic and sessile populations of *Magnusiomyces capitatus* clinical isolates

Bálint Farkas^{1,2,3}, Fruzsina Nagy^{1,2}, Dávid Balázsi¹, Ilona Csányiné Dóczy⁴, Katalin Kristóf⁵, László Majoros^{1,2}, Renátó Kovács^{1,2}, Zoltán Tóth^{1,2}

¹ Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary ² Medical Microbiology, University of Debrecen, Debrecen, Hungary ³ Doctoral School of Pharmaceutical Sciences, University of Debrecen, Debrecen, Hungary ⁴ Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary ⁵ Institute of Laboratory Medicine, Semmelweis University, Budapest, Hungary

Invasive *Magnusiomyces capitatus* infections are rare, closely associated with hematological malignancies and high crude mortality. The therapeutic options are limited as this species shows intrinsic resistance to echinocandins and variable susceptibility to triazoles. Consequently, the currently advised therapeutic protocol for severe infections is amphotericin B alone or in combination with 5-flucytosine, while triazoles, especially voriconazole can be used as step-down therapy or in case of non-life-threatening infections for susceptible isolates. Nonetheless, knowledge on the pharmacodynamic activity of the currently available agents is extremely limited, especially for newer triazoles posaconazole and isavuconazole. Data are similarly scarce on their combinational activity.

In our study, we compared the activity of amphotericin B, 5-flucytosine, fluconazole, posaconazole, voriconazole and isavuconazole against clinical isolates of *Magnusiomyces capitatus* (n=4) using time-kill methodology at physiologically relevant concentrations. Furthermore, we also assessed the interactions of these agents with each other and with micafungin by checkerboard methodology, both against planktonic and sessile populations, using a modified CLSI protocol and XTT assay, respectively.

Amphotericin B showed fungicidal activity against all of the tested isolates at 1 mg/L in a concentration-dependent manner. In contrast, both triazoles and 5-flucytosine showed fungistatic activity. However, while increasing concentrations of triazoles resulted in notably lower CFU numbers, the activity of 5-flucytosine was similar across the tested concentration range. The pharmacodynamic activity of voriconazole and, interestingly, fluconazole was more pronounced than that of posaconazole and isavuconazole resulting in markedly lower CFU counts at 48h. Against planktonic populations, no interaction was observed between the tested agents. In contrast, against biofilms, micafungin showed synergistic activity with triazoles in an isolate-dependent manner.

Our results indicate that the current therapeutic recommendations are supported by the notably better pharmacodynamic activity of voriconazole and amphotericin B in contrast to that of posaconazole and isavuconazole. The positive interaction of micafungin with triazoles against *Magnusiomyces capitatus* biofilms may warrant further investigation.

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Habitat preference of truffle (*Tuber*) species differ among Pannonian forest types

József Geml¹, István Bagi², József Sulyok³

¹Research and Development Centre, Eszterházy Károly Catholic University, Eger, Hungary ²Soil Map Ltd., Taksony, Hungary

³Bükk National Park Directorate, Eger, Hungary

In this study, we carried out ITS rDNA metabarcoding of soil samples collected at 62 sites representing 11 Pannonian forest types to investigate the habitat preferences of *Tuber* species along environmental gradients.

We found 17 different species-level groups of *Tuber* with unique Species Hypothesis codes, based on comparisons of our sequences with curated reference sequences in the UNITE database (unite.ut.ee). These corresponded to 10 known *Tuber* species, most of which had >98% sequence similarity to our sequences. Species of gastronomic and economic value were *T. aestivum*, *T. borchii*, and *T. brumale*, while other species included *T. excavatum*, *T. fulgens*, *T. puberulum*, *T. rapaeodorum*, and *T. rufum*. In the case of two species we found, the most similar reference sequences had relatively low (ca. 95%) sequence similarity and these have no publish records in Hungary to our knowledge: *T. bomiense* and *T. mexiusanum*. Further studies are needed to clarify the taxonomic identities of our sequences that are related to these two species.

Regression analyses revealed that the diversity of *Tuber* species was strongly influenced by environmental factors: we observed strong positive correlations between *Tuber* haplotype diversity and soil pH as well as calcium content, and strong negative correlation with soil moisture content. While downy oak (*Corno-Quercetum*), oak-hornbeam (*Carici pilosae-Carpinetum*), and submontane beech (*Melittio-Fagetum*) forests had the highest number of *Tuber* haplotypes, we did not find significant differences among the eleven Pannonian forest types using analysis of variance. Species composition of truffles differed significantly among forest types, particularly between oak- and beech-dominated forests. Among the measured variables, organic matter, pH, and moisture drive these differences.

Overall, as part of the first comprehensive fungal diversity assessment in a wide range of Pannonian forests based on DNA metabarcoding, the data discussed here indicate that the distribution of *Tuber* species is influenced by niche-based environmental filtering along edaphic and mesoclimatic gradients.

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Functional study of the long control region of human papillomavirus 52

Eszter Gyöngyösi¹, Brigitta László¹, Anita Szalmás¹, József Kónya¹, György Veress¹

¹ Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary

The long control region (LCR) of human papillomaviruses (HPVs) is responsible for regulating viral replication and gene expression. The LCR contains numerous cellular transcription factor binding sites, and its regulatory role is essential for the functions of the HPV E6 and E7 oncoproteins and for viral carcinogenesis. This region of the viral genome is often used to characterize intratype diversity and variant lineages within different HPV types. In the case of high-risk human papillomaviruses, sequence variants may differ in terms of viral persistence and oncogenicity, which can be significantly influenced by sequence differences in the viral LCR. We aimed to investigate the sequence variants of HPV 52, a relatively rare high-risk HPV type in Hungary. To this end, we previously performed a phylogenetic analysis of the HPV 52 intratypic variants identified in Hungary based on LCR sequences. In the present study, we sought to investigate the functional differences between naturally occurring HPV 52 variants using their LCR sequences.

Eighteen specimens, collected from the uterine cervix of women with cytological and/or colposcopic abnormalities, were selected for sequencing and phylogenetic analysis. Among these specimens, 10 distinct variants were identified. According to the phylogenetic tree, all of our variants belonged to lineage A, with 17 samples belonging to sublineage A1 and only one to sublineage A2. We then performed transient transfection experiments to functionally characterize the LCR variants of HPV 52. For this purpose, the complete LCR sequences representing the different HPV 52 lineages and sublineages (A1, A2, B2, C2, and D) and deletion mutants, generated from full-length LCR constructs, were synthesized and cloned into a promoterless luciferase reporter vector. The transcriptional activity of the prototype LCR sequences and their deletion mutants was assessed using luciferase assays.

In functional assays, sublineages A1, A2, and C2 exhibited very similar transcriptional activities, whereas sublineage B2 showed increased activity and lineage D showed reduced activity compared with sublineage A1. We then examined the transcriptional activity of the A1 and B2 deletion constructs and found that the difference in activity was still detectable in those constructs that retained the central enhancer region of the HPV 52 LCR. Furthermore, we investigated potential transcription factor binding sites using relevant databases to identify mutations that might account for the differences in LCR activity among the HPV 52 sublineages. Nucleotide variation in the HPV 52 LCR and alterations in transcription factor binding sites may lead to differences in transcriptional activity among intratypic variants. Phylogenetic and functional analyses together provide a basis for understanding the relationship between LCR polymorphisms and the oncogenic potential of HPV 52 variants.

Glyphosate-induced gene expression changes leading to imipenem-resistance in *Pseudomonas aeruginosa*

Judit Háhn¹, Renáta Ábrahám², Erna Vásárhelyi³, Sándor Szoboszlay¹, Edit Kaszab¹, Erzsébet Baka²

¹Department of Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, 2100, Páter K. street 1., Hungary

²Department of Molecular Ecology, Hungarian University of Agriculture and Life Sciences, Gödöllő, 2100, Páter K. street 1., Hungary

³Department of Environmental Toxicology, Hungarian University of Agriculture and Life Sciences, Gödöllő, 2100, Páter K. street 1., Hungary

The widespread agricultural use of glyphosate-based herbicides (GBHs) leads to sublethal exposure of non-target environmental and opportunistic pathogenic microorganisms, potentially driving antibiotic cross-resistance. Our previous phenotypic studies demonstrated that sublethal exposure to pure glyphosate acid (GLY) and commercial GBH formulations induces specific phenotypic imipenem resistance in *Pseudomonas aeruginosa*, while susceptibility to other carbapenems (meropenem, doripenem) remains unaltered. However, the molecular mechanisms underlying this phenomenon remained uncharacterized. In this study, we investigated the transcriptomic alterations induced by glyphosate (GLY), as well as the combined exposure to glyphosate and imipenem (GLY+IMI).

Global transcriptomic profiling revealed a moderate baseline adaptive response in wild-type cells treated with glyphosate alone (WT vs GLY, $\log_2FC \approx \pm 1-2$). This condition was characterized by the upregulation of genes involved in oxidative stress defence, proteostasis, and amino acid catabolism (including tryptophan degradation), such as anthranilate 1,2-dioxygenase (*antA*, PA50071_12460, $\log_2FC = 2.92$), which was confirmed via RT-qPCR validation. This suggests that glyphosate induces a targeted, specific transcriptional response rather than acute toxic shock.

Upon subsequent imipenem exposure in glyphosate treated cells (GLY vs GLY+IMI), a drastic transcriptomic shift occurred ($\log_2FC$ ranging from +8.4 to -5.8). We observed strong induction of critical resistance determinants, including beta-lactamase/D-alanine carboxypeptidase (*ampC*, PA50071_04165, $\log_2FC = 8.41$) involved in beta-lactam cleavage and cell wall restructuring, as well as the envelope stress-induced protein *YgiW* (PA50071_01575, $\log_2FC = 5.84$), both validated by qPCR. Concurrently, non-essential energy-demanding pathways, such as antibiotic biosynthesis monooxygenase (PA50071_22060, $\log_2FC = -5.78$, qPCR-validated), were profoundly repressed. The direct comparison between the untreated wild-type and the combined treatment (WT vs GLY+IMI) demonstrated the most severe physiological reprogramming ($\log_2FC$ +8.5 to -6.8), reflecting a global resource reallocation toward cell envelope maintenance and survival, accompanied by the complete shutdown of flagellar motility and type VI secretion systems.

Our findings demonstrate that environmental glyphosate exposure acts as a transcriptional priming agent in *P. aeruginosa*. By pre-activating overlapping stress and envelope defence pathways, glyphosate facilitates rapid and potent induction of beta-lactamase activity upon carbapenem challenge. This phenomenon highlights the potential of non-antibiotic environmental pollutants to potentiate multidrug resistance phenotypes in opportunistic pathogens.

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The role of the migratory bird in spreading antimicrobial resistance

Narhulan Halimbek^{1,4,5}, Mihály Mándi¹, Sofia Rizzi¹, Ákos Monoki², Fruzsina Nagy³, Ben Pascoe⁴, Frances Colles⁴, Matthew Hitchings⁶, József Kónya³, Samuel K. Sheppard⁴ & Tamás Székely^{1,5}

¹ Department of Evolutionary Zoology and Human Biology, University of Debrecen, Hungary, ² Ranger Service, Hortobágy National Park Directorate, Debrecen, Hungary, ³ Medical Microbiology, University of Debrecen Clinical Centre, Hungary, ⁴ Ineos Oxford Institute, Department of Biology, University of Oxford, UK, ⁵ Centre for Evolution, Department of Life Sciences, University of Bath, UK, ⁶ Institute of Life Science, Swansea University, UK

Antimicrobial resistance (AMR) is increasingly recognised as an ecological issue that extends beyond clinical environments. Resistant bacteria and resistance genes have been detected in wildlife, including species with no direct exposure to antibiotics, highlighting the value of wild animals as sentinels of environmental AMR. Migratory shorebirds are particularly relevant because they use aquatic habitats, interact with diverse environments and move across large geographic scales. In this project, we investigate how bird ecology and migration may shape AMR connectivity across wildlife, domestic animals and humans. Sampling in Hungary includes wild shorebirds, poultry farms and clinical settings, followed by DNA extraction and shotgun metagenomic sequencing. This approach allows us to characterise microbial communities and provides the basis for comparing AMR profiles across host groups and environments. Here, we present the first metagenomic results from shorebird faecal samples. These initial analyses describe the broad taxonomic composition of sequencing libraries and genus-level bacterial community diversity across sampled shorebird species. These data establish an ecological and microbiological baseline for subsequent analyses of antimicrobial resistance, enabling future comparisons among wild birds, poultry and humans and helping us assess how migratory birds may contribute to AMR connectivity across environments and geographic regions.

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From Colonization to Sterile-Site Infections: Clinical and Therapeutic Aspects of *Saccharomyces cerevisiae*

Andrea Harmath^{1,2}, Ágnes Jakab^{1,4}, Zoltán Tóth^{1,4}, Aliz Bozó^{1,4}, László Majoros^{1,4}, Valter P. Pfliegler³, Renátó Kovács^{1,4}

¹Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

²Doctoral School of Pharmaceutical Sciences, University of Debrecen, Debrecen, Hungary

³Department of Molecular Biotechnology and Microbiology, University of Debrecen, Debrecen, Hungary

⁴Medical Microbiology, Clinical Centre, University of Debrecen, Debrecen, Hungary

Saccharomyces cerevisiae has been increasingly identified as an opportunistic colonizer of the gastrointestinal tract and as an emerging cause of clinically significant infections in hospitalized patients. In this retrospective study, *S. cerevisiae* isolates were collected from sterile body sites at the University of Debrecen Clinical Centre between January 2019 and December 2025. Of 196 patients with *S. cerevisiae*-positive clinical samples, 30 (15%) had isolates recovered from sterile body sites. The cohort included 16 females and 14 males, with a median age of 47.2 years (range, 0–84); seven patients (23%) were younger than 18 years. Bloodstream infections were most frequent (23%), followed by intra-abdominal abscess aspirates and drained intra-abdominal wound samples (17% each). Concomitant bacterial pathogens were identified in 77% of cases, most commonly *Acinetobacter* spp., *Klebsiella pneumoniae*, *Enterococcus* spp., and *Pseudomonas aeruginosa*. Previous gastrointestinal surgery was documented in 63% of patients, 33% required intensive care, and 33% had exposure to *S. cerevisiae* var. 'boulardii'-containing probiotics. Antifungal therapy was administered in 63% of cases, most frequently with fluconazole or anidulafungin. The 30-day mortality rate was 27%. Increasing age ($p=0.008$) and intensive care unit admission ($p=0.03$) were independent predictors of mortality. Antifungal susceptibility testing demonstrated substantial minimum inhibitory concentration (MIC) variability, with fluconazole MICs ranging from 0.125 to 128 µg/mL (median, 8 µg/mL). Amphotericin B exhibited predominantly low MICs (median, 0.25 µg/mL), while anidulafungin showed the lowest MIC values (median, 0.008 µg/mL). These findings highlight the clinical relevance of *S. cerevisiae* recovered from sterile body sites and identify gastrointestinal surgery, intensive care, and probiotic exposure as frequent clinical features among affected patients. The observed variability in antifungal susceptibility underscores the necessity for thorough clinical assessment and individualized therapeutic strategies.

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Proposal for a new virus family and order

Balázs Harrach¹, Thomas S. Postler², Mart Krupovic³

¹ HUN-REN Veterinary Medical Research Institute, Budapest, Hungary, ² Vaccine Design and Development Lab, IAVI, Jersey City, NJ, USA, ³ Institut Pasteur, Université Paris Cité, Cell Biology and Virology of Archaea Unit, Paris, France

A partial viral genome labelled as adenovirus (AdV) originating from a spider has been deposited to GenBank. AdVs in invertebrates? We identified the genomes of three additional related viruses: a virus from a bird, named “AdV”, one from another spider, named “midsize eukaryotic linear dsDNA (MELD) virus”, and one from a fish, labelled as “Cetobacterium”. These three complete/coding-complete genomes and one partial genome have been recovered by metagenomics from chestnut-rumped thornbill faeces (BK066456), tangle-web spider (BK012043), yellow catfish gut (JBOGBR010000001) and crab spider (BK066476). The thornbill and the crab spider viruses were labelled in sequence databases as “AdV”. These sequences display remote similarity to those of AdV. For instance, the order of the genes encoding DNA polymerase (*pol*), penton base, hexon and protease on the genome is similar to that in AdVs. However, these viruses are markedly different from the members of the *Adenoviridae* in genome organization, pairwise distance and phylogeny. In a global maximum likelihood phylogenetic analysis of protein-primed family B DNA polymerases (pPolB) encoded by diverse eukaryotic viruses and plasmids, these unclassified viruses formed a sister group to the clade including classified AdVs. Thus, we proposed classifying these viruses into a new family, “*Paradenoviridae*”, to be placed into a new order, “*Fictadevirales*”, a sister to *Rowavirales*.

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Challenges and possibilities in virus taxonomy

Balázs Harrach¹, Győző L. Kaján¹, Márton Z. Vidovszky¹, Mária Benkő¹, Andor Doszpoly¹

¹ HUN-REN Veterinary Medical Research Institute, Budapest, Hungary

The analysis of a virus genome is only finished when its sequence is submitted to the International Committee on Taxonomy of Viruses (ICTV) for classification into a new species. If the virus does not belong to an existing genus, a proposal is needed also for establishment of a new genus, because the obligatory binomial species names are formed from a genus name followed by an epithet. The style of epithets is highly variable in different families, e.g., circovirid species epithets include the host name in the language spoken in the region where the corresponding virus was identified (including Hungarian, with a challenging example being *Circovirus gyurgyalag*). Adenovirus (AdV) epithets may include Latin/Latinized host name, disease, geographical location, scientists' names, numbers (referring to types) or colours (for parrot AdVs). Some families have free-form epithets consisting of text and Arabic number combinations (e.g., *Simplexvirus humanalpha1*). The guidelines for species naming can be found on the ICTV website (ictv.global), whereas the Virus Metadata Resource lists not only the binomial species names but also the names of the member viruses. The demarcation criteria for species differ across the families but usually are based on amino acid (sometimes nucleotide) sequence, phylogenetic analyses, and/or pairwise nucleotide identity thresholds. Classification of viruses to the higher taxonomic ranks (order, class, etc.) can be more challenging.

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First screening of *Francisella tularensis* among patients with severe acute respiratory infections in Hungary

Panna Hauzman-Sütő¹, Norbert Kiss¹, Bereniké Novák¹, Gyula Zsidei¹, Judit Henczkó¹

¹Department of National Biosafety Laboratory, National Center for Public Health and Pharmacy, Budapest, Hungary

Francisella tularensis, the causative agent of tularemia is a zoonotical, intracellular bacteria responsible for rare but potential severe disease. *F. tularensis* classified as category A pathogen and comprises four subspecies, of which subspecies *tularensis* (type A) and *holarctica* (type B) are the most clinically significant. In Europe, including Hungary, *F. tularensis* subsp. *holarctica* is endemic and accounts for the majority of locally acquired infections. Clinical presentations of tularemia depending on its transmission route: glandular, ulceroglandular, oculoglandular, oropharyngeal, typhoid, or pneumonic. In Hungary, fewer than 100 cases are reported annually. Early signs often resemble non-specific flu-like symptoms and lymphadenopathy then may develop these forms. Furthermore, the diagnostic challenge compounded by the limitations of traditional culture-based methods, which require BSL-3 containment.

National Center for Public Health and Pharmacy (NCPHP, NNGYK) operates a nationwide surveillance system for respiratory viral infections. For this retrospective study, respiratory specimens collected between 2024 and 2026 were selected from the surveillance system, with a particular focus on patients with severe acute respiratory infections (SARI), to determine the frequency of pneumonic tularemia in Hungary. In total n=971 samples were analysed. Nucleic acid isolation was performed using the Roche MagNA Pure 96 system with the DNA and Viral RNA small volume kit. Samples were screened for *F. tularensis* using an in-house real-time PCR assay targeting the *tul4* gene. The diagnostic algorithm included characterisation of positive samples using an in-house real-time PCR assay targeting the ISFtu2 element. One of the 971 respiratory samples tested positive and was identified as *F. tularensis* subsp. *holarctica*.

Based on data from Hungary and other European countries, the ulceroglandular and glandular forms are the most common clinical manifestations of tularemia, whereas the pneumonic form considered rare. The detection of one positive sample among the 971 samples tested (0,10%) is consistent with the currently available epidemiological data and our previous experience. It highlights the potential for pneumonic tularemia to remain under-recognised among patients presenting with severe respiratory illness, particularly in endemic regions where routine diagnostic algorithms may not initially include *F. tularensis*. Incorporating targeted molecular screening into established respiratory surveillance platforms may provide an additional mechanism for detecting sporadic or atypical tularemia cases and contribute to improved epidemiological surveillance. Successful management of tularemia depends on early clinical recognition, timely laboratory diagnosis, and the administration of appropriate antimicrobial agents. This work was supported by the HERA2 project, co-funded by the European Union under the EU4Health Programme (grant agreement No. 101102440).

Biomass Production and Rhizosphere Microbial Communities of Szarvasi-1 Energy Grass Cultivated on Dredged Sediment

Flóra Boglárka Horváth^{1,2,3}, Barbara Téglás⁴, Ábel Németh^{4,5}, Gyula Sipos⁶, Ferenc Fodor^{2,3}, Erika Tóth^{1,5,7}

¹ Department of Microbiology, Eötvös Loránd University, Budapest, Hungary, ² Doctoral School of Biology, Eötvös Loránd University, Budapest, Hungary, ³ Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, Budapest, Hungary, ⁴ National Center for Public Health and Pharmacy, Budapest, Hungary, ⁵ Doctoral School of Environmental Sciences, Eötvös Loránd University, Budapest, Hungary, ⁶ Agricultural Research and Development Institute, Szarvas, Hungary, ⁷ Research and Development Center, Eszterházy Károly Catholic University, Eger, Hungary

In this study, we evaluated the suitability of dredged sediment from Lake Balaton as a growing medium for Szarvasi-1 energy grass. In addition, the effects of fertilizer and microbial inoculum treatments were assessed, and changes in the rhizosphere prokaryotic community were monitored throughout plant development.

A container experiment was conducted under field conditions using forty-five 1 m³ containers filled with dredged sediment and five control containers filled with soil. Biomass yield was determined during summer harvests. Rhizosphere samples were collected from thirty containers on four occasions (autumn 2023, winter 2024, summer 2024, summer 2025) and analyzed by amplicon sequencing.

The stand showed stable biomass production during the first year (2024; mean 1.4101 kg per container), with higher yields in sediment treatments than in the control soil. By the second year, productivity declined in all cases (2025; mean 0.5980 kg per container). The inoculum treatments did not result in significant differences in biomass production.

Microbiological analyses revealed a gradual restructuring of the prokaryotic community. Community diversity increased markedly by the first summer after sowing and decreased moderately by the second summer (Shannon diversity index winter 2024: 2.325-6.626; summer 2024: 4.883-7.013; summer 2025: 5.361-6.578). Between the samples collected in autumn 2023 and winter 2024, the relative abundance of the phylum *Patescibacteria* increased, whereas that of *Desulfobacterota* decreased. Compared to the original dredged sediment, this shift was most likely attributable to the rhizosphere effect. Throughout the entire study period, members of the phyla *Actinomycetota* and *Pseudomonadota* exhibited dominant occurrence within the microbial communities. During plant development, the genera *Nocardioidea* and *Streptomyces* became predominant within the phylum *Actinomycetota*. Several representatives of these genera are known to possess plant growth-promoting (PGP) properties. Within the phylum *Pseudomonadota*, members of the families *Xanthobacteraceae*, *Comamonadaceae*, *Sphingomonadaceae*, and *Lysobacteraceae* were detected at high relative abundances. The observed changes in the heterotrophic microbial community are likely associated with alterations in nutrient availability and composition within the rhizosphere. Members of the domain *Archaea* were also identified in the 2025 samples. The community was predominantly composed of representatives of the class *Nitrososphaeria*, of the phylum *Thermoproteati*, which are recognized as important contributors to nitrogen cycling.

Overall, no treatment-dependent differentiation of the microbial communities was observed. The community was dominated by organic matter-degrading taxa and microorganisms with potential PGP capabilities.

Construction and phenotypic characterization of *Beauveria bassiana* Bbsod3 and BbatfA deletion and complemented mutants by *Agrobacterium tumefaciens*-mediated transformation

Sandugash Ibragimova^{1,2}, Beatrix Kocsis^{1,2}, Éva Leiter^{1,2}, István Pócsi^{1,2}

¹ Department of Molecular Biotechnology and Microbiology, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary, ² HUN-REN-UD Fungal Stress Biology Research Group, Debrecen, Hungary

Beauveria bassiana is an entomopathogenic fungus widely used as a biocontrol agent against agricultural insect pests. Its field persistence and efficacy depend on virulence and resistance to environmental stressors, including high temperatures, solar UV radiation, drought, and agrochemicals. Manganese-dependent superoxide dismutases (MnSODs) are key components of the fungal antioxidant defense system, catalyzing the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen. The cytosolic MnSOD BbSod2 contributes to oxidative stress tolerance, UV resistance, and virulence in *B. bassiana*. In contrast, the biological role of the mitochondrial manganese SOD isoenzyme Bbsod3 has remained poorly understood. The bZIP transcription factor AtfA is a conserved master coordinator of fungal developmental and physiological preparedness. Investigating the function of BbSod3 and BbAtfA is essential for understanding the environmental adaptation of *B. bassiana*. In this study, *Agrobacterium tumefaciens*-mediated transformation (ATMT) method was used to generate $\Delta Bbsod3$ and $\Delta BbatfA$ deletion mutants together with their complemented strains. The *bar* resistance gene was used as a selection marker for gene deletion, whereas the *sur* gene served as a selection marker for the complementation. Wild-type, deletion mutant and complemented strains were tested for their responses to oxidative and osmotic stress by measuring colony growth. $\Delta Bbsod3$ showed no growth in the presence of 0.2 mM menadione sodium bisulfite (MSB) and $\Delta BbatfA$ strain had decreased colony growth in response to 0.8 mM *tert*-butyl hydroperoxide. Complementation restored the WT phenotype in both mutants, confirming that the stress-sensitive phenotypes were directly attributable to the targeted gene deletions. These results highlight the reliability of ATMT for genetic modification of *B. bassiana* and show distinct roles of BbSod3 and BbAtfA in oxidative stress adaptation of this fungus. BbSod3 is essential in the protection against MSB-generated superoxide radicals and BbAtfA has a role in resistance to organic peroxide stress.

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

Ágnes Jakab^{1,2}, Fruzsina Kovács^{1,2}, Andrea Harmath^{1,3}, Dávid Balázs^{1,3}, Lajos Forgács¹, Zoltán Tóth^{1,2}, András Babják¹, Flórián Szatmáry¹, Aliz Bozó^{1,2}, László Majoros^{1,2}, Renátó Kovács^{1,2}

¹ Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary ² Medical Microbiology, University of Debrecen, Debrecen, Hungary ³ Doctoral School of Pharmaceutical Sciences, University of Debrecen, Debrecen, Hungary

Candida auris is an emerging multidrug-resistant fungal pathogen associated with healthcare-associated outbreaks and persistent colonization. Its ability to form biofilms contributes to increased tolerance to antifungal agents and represents a major therapeutic challenge. In this study, we investigated the activity of caspofungin and posaconazole, alone and in combination, against biofilms formed by South Asian clade *C. auris* isolates, and explored the molecular responses associated with combined antifungal exposure.

Six *C. auris* isolates were investigated using biofilm susceptibility assays and metabolic activity measurements. The interaction between caspofungin and posaconazole was assessed using checkerboard assays, while the effect of the combination was further evaluated in an *in vivo* model. Transcriptomic analysis was performed to characterize the cellular response of *C. auris* biofilms to antifungal treatment.

The caspofungin–posaconazole combination demonstrated synergistic activity against all tested *C. auris* biofilm isolates, with fractional inhibitory concentration indices ranging from 0.078 to 0.21. Compared with monotherapy, the combination resulted in substantial reductions in the median MICs of both caspofungin and posaconazole. The synergistic effect was also confirmed *in vivo*. Transcriptomic analysis revealed an extensive response to combined antifungal stress, including activation of stress-response pathways and remodelling of the fungal cell wall and plasma membrane. Changes in chitin, the mannan–glucan complex, sphingolipid and ergosterol metabolism, and intracellular ion homeostasis were observed. Increased expression of genes associated with drug efflux and cellular stress responses, including *MDR1* and *RCT1*, further indicated adaptive mechanisms supporting fungal survival.

Our findings demonstrate the potent synergistic activity of caspofungin and posaconazole against *C. auris* biofilms and provide insights into the molecular mechanisms underlying the response to combination therapy. These results support further investigation of this combination as a potential strategy for biofilm-associated infections caused by multidrug-resistant *C. auris*.

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Investigation of how temperature modulates antiviral RNAi, virus accumulation, and detectability of viruses

Nikoletta Jaksa-Czotter¹, Éva Várallyay¹

¹Genomics Research Group, Department of Plant Pathology, Institute of Plant Protection, Hungarian University of Agriculture and Life Sciences, Hungary, H-2100 Godollo, Szent-Györgyi A. Street 4.

Antiviral RNA interference (RNAi) is a natural defence mechanism against viruses in plants, mediated by the production of virus-derived small interfering RNAs (siRNAs; 21–24 nt) that target virus RNA through sequence complementarity. While environmental factors such as temperature influence plant–pathogen interactions, the impact of these factors on viral replication, antiviral RNAi, and the consequences for virus detection has not been well characterised in herbaceous plants or studied in woody hosts. In our study, we investigated how temperature and its fluctuations modulate virus concentrations and the antiviral RNAi responses in plum pox virus (PPV)-infected herbaceous and economically important woody plants.

PPV-infected *Nicotiana benthamiana* was maintained at controlled temperatures of 15°C, 20°C, and 28°C, while graft-inoculated PPV-infected plum were grown inside and outside of an isolator house under natural conditions. Virus accumulation and molecular detectability were tested using RT-PCR and RT-qPCR, while characteristics of antiviral RNAi were determined using small RNA high-throughput sequencing (sRNA-HTS).

Our results showed that, in *N. benthamiana*, PPV accumulation showed a pronounced temperature dependence, with high viral RNA levels at 20°C, lower accumulation at 28°C, and a dramatic reduction at 15°C, while temperature did not affect the total sRNA size distribution, with 24 nt sRNAs predominating at all temperatures.

Temperature showed very big fluctuations and peaks under natural conditions. As a consequence of pronounced daily temperature fluctuations, higher virus titres were measured in the open field-grown grafts compared with the ones maintained in the isolator. The sRNA profile of the grafts was very different from that observed in *N. benthamiana*, 21 nt sRNAs were the predominant size. The concentration of vsiRNAs, particularly 21–22 nt species, was also higher under open field conditions. More importantly, the graft in the open field had PPV-specific symptoms, and the virus could be detected in them with a serological test, while the graft in the isolator was symptomless and negative for the test. Overall, our results show that temperature and daily temperature fluctuations can strongly affect antiviral RNAi and virus accumulation. Changes in virus titre can influence virus detectability and the sensitivity of diagnostic assays. These findings highlight the importance of considering environmental conditions in plant virus diagnostics and phytosanitary surveillance.

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Development of an online service for automated molecular identification of adenovirus species and types

Győző L. Kaján, Balázs Harrach

HUN-REN Veterinary Medical Research Institute, Budapest, Hungary

Bioinformatic analysis of adenoviral sequences can be challenging and time-consuming, particularly when species identification requires phylogenetic analysis with reference strains. The International Committee on Taxonomy of Viruses (ICTV) recommends adenovirus species demarcation based on phylogenetic distance, with a threshold of 10–15% in maximum-likelihood analyses of the DNA polymerase (pol) amino acid sequence. We aimed to develop an automated, user-friendly service for rapid adenovirus species identification and molecular typing.

The service was developed in Python using an AI-assisted software development approach. A curated reference database was assembled containing DNA polymerase amino acid sequences of adenoviral ICTV exemplar strains, with current and officially recognised binomial species names. A second curated nucleotide database containing adenovirus types was established for molecular typing within species; type nomenclature and numbering followed the Adenoseq resource (sites.google.com/site/adenoseq). The workflow accepts nucleotide sequences in FASTA format or GenBank accession numbers. The pol open reading frame (ORF) is automatically identified and translated, and the resulting amino acid sequence is compared with the species reference database using BLASTx. For molecular type identification, nucleotide sequences are compared with the type reference database using BLASTn. Candidate sequences are subsequently subjected to multiple sequence alignment and maximum-likelihood phylogenetic analysis.

Based on amino acid sequence identity, the pipeline assesses whether a submitted sequence is consistent with an established species or may represent a potentially novel species. In parallel, BLASTn analysis against the curated type database enables rapid molecular identification of the adenovirus type within the assigned species. The workflow also provides the automated reconstruction and visualisation of a phylogenetic tree. The service is continuously available through a web-based Streamlit interface at advtyping.vMRI.hun-ren.hu.

The service provides a practical tool for rapid adenovirus classification at both species and molecular type levels. Applications include diagnostic investigations, exploration of potentially novel adenoviruses and high-throughput typing of multiple strains. Automatic identification of the pol ORF allows complete genome sequences to be analysed without prior annotation. The combination of automated species-level assessment, molecular type identification and phylogenetic analysis in a single workflow may facilitate reproducible and efficient adenovirus characterisation.

We gratefully acknowledge the HUN-REN Cloud infrastructure for providing computational resources. This work was supported by the National Research, Development and Innovation Fund (NKFIH), grant K146808.

First record of methyl N-hydroxybenzimidate production by the antagonistic yeast *Metschnikowia pulcherrima* and its potential in bioprotection.

Zoltán Kállai ¹, Matthias Sipiczki. ^{1,2}

¹ Institute of Horticultural Science, University of Debrecen, Debrecen, Hungary ² Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary

In 2006, pigment-producing *Metschnikowia* yeasts isolated from noble rot-infected grapes in our laboratory were reported to display antagonistic activity against other microorganisms (1). It was proposed that these pigmented *Metschnikowia* isolates suppressed the growth of sensitive microorganisms by producing and secreting pulcherriminic acid. This cyclic dipeptide depletes the free iron in the medium by complexing the ferric ions in the water-insoluble pigment pulcherrimin. Our research on *Metschnikowia* yeasts has continued without interruption. We have gained deeper insight into the antagonistic mechanism underlying pulcherriminic acid production and its genetic background. In a recent study, we analysed the fermentation broths of antagonistic *Metschnikowia* strains using gas chromatography-mass spectrometry, and found that they produced methyl N-hydroxybenzimidate in significant quantities. This antimicrobial compound was first described from a *Streptomyces pratensis* strain in 2017 (2). Its production has since been reported in several *Bacillus* species, as well as in *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii*. The compound has already been shown to act against both Gram-positive and Gram-negative bacteria, including some methicillin-resistant strains (2, 3), and a 2025 study suggested that it might also be effective against *Fusarium oxysporum* (4). Inspired by these findings, we began testing this substance for antimicrobial effects against microorganisms relevant to food safety.

Acknowledgments

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Screening of rhizosphere-derived microorganisms for plant growth-promoting traits

Orsolya Kedves¹, Emese Judák¹, Henrietta Allaga¹, András Varga¹, Ingrid Melinda Gyalai², Flórián Kovács², Csaba Vágvölgyi¹, László Kredics¹

¹Department of Biotechnology and Microbiology, Institute of Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary ² Institute of Plant Sciences and Environmental Protection, Faculty of Agriculture, University of Szeged, Hódmezővásárhely, Hungary

Rhizosphere-associated microorganisms are increasingly recognized as important contributors to plant nutrition, growth and adaptation to environmental stress. Their beneficial effects may involve several mechanisms, including nutrient mobilization, iron acquisition and the production of plant growth-regulating compounds. However, the functional potential of culturable rhizosphere microorganisms can vary considerably among isolates and host plants, making systematic screening an important step in the selection of microorganisms for agricultural applications.

In the present study, culturable microorganisms were isolated from the rhizosphere of five economically important crop species: lettuce (*Lactuca sativa*), wheat (*Triticum aestivum*), tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*) and cucumber (*Cucumis sativus*). Twenty bacterial isolates were selected from each plant species, resulting in a collection of 100 bacterial isolates. In parallel, approximately 20–25 fungal isolates were recovered using *Trichoderma*-selective cultivation. Molecular identification is combined with phenotypic and functional characterization to obtain a comprehensive description of the selected rhizosphere-derived microorganisms.

The bacterial isolate collection is screened for agriculturally relevant plant growth-promoting traits, including phosphate mobilization, siderophore production and indole-3-acetic acid production. These assays are used to compare the functional potential of microorganisms originating from different crop rhizospheres and to identify isolates possessing several potentially beneficial characteristics. The integration of molecular and functional data enables a more detailed evaluation of the diversity and agricultural relevance of the isolate collection.

The study provides a structured basis for selecting promising rhizosphere-derived microorganisms for subsequent plant-based validation. By linking crop origin, taxonomic identity and experimentally determined functional properties, the work contributes to a better understanding of culturable plant-associated microbiota and supports the development of biologically based approaches aimed at improving nutrient acquisition and sustainability in crop production.

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Exploring relationships among grapevine physiology and berry and leaf nutrient and mycobiome composition in Kékfrankos scion grafted on different rootstocks

Glodia Kgobe¹, József Geml¹, Adrienn Geiger¹, Anna Molnár¹, Carla Mota Leal¹, Adrienn Tóth², Szabolcs Villangó², Lili Mézes¹, Zsolt Zsófi²

¹Research and Development Centre, Eszterházy Károly Catholic University, Eger, Hungary ²Institute for Viticulture and Enology, Eszterházy Károly Catholic University, Eger, Hungary

The main objective of this study was to explore the influence of rootstock genotype on the scion berry and leaf mycobiome, nutrient content, and physiological activities. Some of the major factors that affect the performance of grapevine include vigor conferred by the rootstock. We conducted fungal DNA metabarcoding of berry and leaf samples collected in August and September 2020 from the Nagy-Eged Hill terroir in Northern Hungary from grapevines of the same scion cultivar (Kékfrankos) grafted on three rootstocks that differ in their induced scion vigor. Kékfrankos is considered a non-osmoregulating cultivar, while the rootstock Georgikon 28 is suggested to induce low vigor, whereas Teleki 5C and 125 AA are considered to induce medium to high vigor. We statistically compared the composition, richness, and abundance of taxonomic and fungal functional groups among the rootstocks.

Results we obtained from leaf and berry chemical properties and mycobiome composition, as well as from leaf physiological measurements, concordantly indicate significant effects of rootstock genotype influence on the scion leaf and berry mycobiome composition, performance, biochemical processes, and resulting crop properties of the same scion under growing conditions characterized by chronic moderate water stress. In addition, we observed significant correlations between species turnover of leaf-associated fungi and differences in leaf stomatal conductance among samples, but not in assimilation rate. While the effects of rootstock on scion physiology are well-known from the literature and are corroborated by our measurements, the correlation between scion leaf physiological parameters and leaf-associated fungal community composition is novel.

The emerging picture highlights the existence of features driven by rootstock–scion interactions in shaping the grapevine mycobiome. Despite the observed correlations between leaf mycobiome composition and leaf physiological parameters, we cannot determine a direct causal relationship, based solely on the current results and, therefore, further investigation is warranted.

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Preclinical evaluation of a measles-vectored Nipah virus glycoprotein vaccine in a hamster model

Zoltan Kis^{1,2,3}, Tomoko Fujiyuki⁴, Misako Yoneda⁴, Chieko Kai⁴, Dániel Déri^{1,3}, Judit Henczkó^{1,3}, Nóra Deézsi-Magyar^{1,3}, Bereniké Novák^{1,3}, Gyula Zsidoi^{1,3}, Bernadett Pályi^{1,3}

¹ Department of National Biosafety Laboratory, National Center for Public Health and Pharmacy, Budapest, Hungary

² Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary

³ Hungarian node of European Research Infrastructure on Highly Pathogenic Agents (ERINHA), Budapest, Hungary

⁴ The Institute of Industrial Science, The University of Tokyo, Tokyo, Japan

Nipah virus (*Henipavirus nipahense*, NiV) is a highly pathogenic, re-emerging zoonotic paramyxovirus. Its high case-fatality rate and potential for human-to-human transmission make NiV a major threat with pandemic potential. Currently, there are no licensed vaccines available. Consequently, the World Health Organization (WHO) has listed NiV as a priority pathogen.

The MV-NiV vaccine candidate—developed by the C. Kai and M. Yoneda group at the University of Tokyo—utilizes the attenuated measles virus (Edmonston strain) expressing the NiV (Malaysia strain) glycoprotein, the primary immunogenic target responsible for receptor binding. Preclinical efficacy was evaluated in 6–8-week-old male and female Syrian golden hamsters (*Mesocricetus auratus*; n=6–8/group). Animals were immunized intraperitoneally with various doses at a three-week interval. To study the effect of pre-existing vector immunity, a subset of animals was pre-immunized with MMRvaxPro (Merck). Homologous (NiV-Malaysia, NiV-M) and heterologous (NiV-Bangladesh, NiV-B) wild-type strains were used for challenge experiments. A subset (30%) of animals was sacrificed at 4–5 days post-infection (dpi) for organ analysis. Survival rate, viral RNA, infectious virus titers, total IgG, and neutralizing antibody responses were assessed.

Both total anti-NiV IgG and serum neutralizing antibody titers showed clear dose dependence. Two high doses of the vaccine fully protected animals from significant weight loss and lethal infection following challenge with either NiV strain. Histopathological lesion scores and organ viral loads were significantly lower in the high-dose group compared to the low-dose and placebo groups on dpi 4–5 ($p < 0.05$) and at the study endpoint ($p < 0.01$). Post-challenge neutralizing antibody levels were higher against NiV-M than NiV-B in the heterologous challenge group. Importantly, pre-existing immunity against the measles vector did not impair vaccine efficacy: animals pre-vaccinated with MMR showed anti-NiV IgG and neutralizing antibody titers comparable to those in the non-pre-immunized MV-NiV group, resulting in complete protection against lethal challenge.

These findings demonstrate that MV-NiV is highly immunogenic and protective against both homologous and heterologous NiV strains, even in the presence of pre-existing vector immunity. This highlights its strong potential as a vaccine candidate for further clinical development.

Investigations of delafloxacin resistance mechanisms in Enterobacterales clinical isolates

Béla Kocsis¹, András Kubicskó¹, Dániel Gulyás², Katalin Kamotsay³, Dóra Szabó¹

1. Institute of Medical Microbiology, Semmelweis University, 1089 Budapest, Hungary, 2. Budapest Uzsoki Hospital, Central Laboratory, 1145 Budapest, Hungary, 3. Central Microbiology Laboratory, National Institute of Hematology and Infectious Disease, Central Hospital of Southern-Pest, 1097 Budapest, Hungary

Abstract

Delafloxacin is a new fluoroquinolone agent that has been introduced into clinical applications. Our study analyzed efficacy of delafloxacin on *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates. Altogether 80 *E. coli* and 43 *K. pneumoniae* were included in this study in Budapest, Hungary. Antimicrobial susceptibility testing was performed by broth microdilution method for ciprofloxacin, delafloxacin, levofloxacin, moxifloxacin, ceftazidime, cefotaxime, imipenem. Selected multidrug-resistant and delafloxacin resistant strains were analyzed by whole genome sequencing (WGS) on Illumina MiSeq platform to detect resistance determinants and sequence types (ST). Based on susceptibility results of *E. coli*, delafloxacin and ciprofloxacin resistance rates were 38.75% (31/80) and 41.25% (33/80), respectively. In case of *K. pneumoniae* delafloxacin and ciprofloxacin resistance rates were 46.5% (20/43) and 51% (22/43), respectively. WGS analysis detected mutations in quinolone resistance determining regions (QRDR) of selected delafloxacin resistant *E. coli* strains namely, mutations in *gyrA* Ser83Leu, Asp87Asn and *parC* Ser80Ile were detected in ST162, ST57 and ST15840 strains. In case of ST131 strains combination of five mutations were detected: *gyrA* Ser83Leu, Asp87Asn, *parC* Ser80Ile, Glu84Val, *parE* Ile549Leu. In ST43 strains *gyrA* Ser83Leu, Asp87Asn, *parC* Ser80Ile, Glu84Val mutations were detected.

In delafloxacin resistant *K. pneumoniae* *gyrA* Ser83Ile and *parC* Ser80Ile mutations were detected in ST307 and ST147 strains. In the ST377 strain, *gyrA* Ser83Tyr, Asp87Ala, and *parC* Ser80Ile mutations were detected. Each delafloxacin-resistant *K. pneumoniae* strain carried OqxAB and AcrAB efflux pumps.

Delafloxacin demonstrates potent antibacterial efficacy against *E. coli* and *K. pneumoniae* clinical isolates. However, multiple QRDR mutations can confer delafloxacin resistance in *E. coli* and *K. pneumoniae*.

High throughput screening for accelerated industrial strain improvement

Zoltán Kondor¹, Andrea Zsuzsanna Ujvárosi¹

¹ TAPI Hungary Industries Ltd., Debrecen, Hungary

Continuous strain improvement is a cornerstone of modern industrial biotechnology, driving increased productivity, enhanced strain robustness, and improved product quality, thereby sustaining the profitability of fermentation-based products.

However, the traditional development approach is time-, space-, and resource-intensive, limiting overall efficiency. To maintain competitiveness, a paradigm shift toward more efficient high-throughput screening (HTS) methods has become essential.

The key element of this approach is the miniaturization of the fermentation process from shake-flask scale to microplate format, combined with automated and robotic systems that provide a robust, efficient, and well-controlled screening workflow. The HTS platform covers the entire process, from colony picking and cultivation to sample preparation, analytical measurement, and data evaluation.

The miniaturization and automation of screening activities not only increase throughput but also reduce the environmental footprint of strain development through lower material consumption, reduced waste generation, and more efficient utilization of laboratory resources.

The presentation highlights how HTS technologies have been integrated into our strain improvement pipeline and how they contribute to faster candidate selection and more efficient development workflows.

New World hantavirus infections: diagnostic challenges and public health lessons from a recent outbreak

Anita Koroknai¹, Orsolya Nagy^{1,2}, Anna Nagy¹, Nikolett Csonka¹, Katalin Szomor¹, Mária Takács^{1,2}

¹Department of Virology, Division of Microbiology Reference Laboratories, National Center for Public Health and Pharmacy, Budapest, Hungary ²Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary

Hantaviruses are rodent-borne zoonotic pathogens that are classified into Old World and New World hantaviruses based on their geographical distribution and clinical manifestations. Old World hantaviruses primarily cause haemorrhagic fever with renal syndrome (HFRS), whereas New World hantaviruses, circulating in the Americas, are responsible for hantavirus cardiopulmonary syndrome (HCPS), a severe disease associated with a high case fatality rate. The recently reported Andes virus outbreak aboard the *MV Hondius* cruise ship has once again highlighted the public health significance of New World hantaviruses. Andes virus is unique among hantaviruses in its ability to spread from person to person, making early recognition, laboratory diagnosis, and contact tracing particularly important.

In Hungary, laboratory diagnosis of hantavirus infections is performed by the National Reference Laboratory for Viral Zoonoses at the National Center for Public Health and Pharmacy. In addition to diagnostic assays for endemic hantaviruses, the laboratory has serological and molecular methods available for the detection of New World hantaviruses.

The presentation provides an overview of the virological and epidemiological characteristics of New World hantaviruses, the clinical features of hantavirus cardiopulmonary syndrome, and the strengths and limitations of current laboratory diagnostic approaches. Serological and molecular diagnostic methods, their appropriate application, and the epidemiological lessons learned from the recent Andes virus outbreak are also discussed.

Although New World hantavirus infections are not endemic in Hungary, imported cases should be considered in the context of increasing international travel and globalization. Early clinical suspicion, appropriate specimen collection, and timely laboratory diagnosis play a key role in confirming infection and supporting the prompt implementation of public health measures.

Aflatoxin B1 degradation experiments with *Rhodococcus* bacterial strains (*Rhodococcus erythropolis* NI86/21, *R. qingshengii* NI2 and *R. jostii* JCM 11615)

Judit Kosztik, József Kukolya, Ildikó Bata-Vidács, Helga Szalontai, István Nagy

Eszterházy Károly Catholic University

Rhodococcus erythropolis NI86/21 is a Gram-positive soil bacterium with exceptionally versatile metabolism, isolated from the maize root zone in Hungary. The strain became widely known in international biotechnological research—particularly through studies conducted at the Belgian university KU Leuven—as a prominent model organism for environmental bioremediation and plant protection technologies.

One of the bacterium's key characteristics is its genome, which exceeds 8 megabase pairs in size—outstanding even within the species—consisting of a large chromosome and five extrachromosomal elements. The 6 kb cryptic plasmid designated pFAJ2600 isolated from this strain was used to develop shuttle vectors capable of replicating in *Escherichia coli* and *Rhodococcus* species. The high degree of genome plasticity, along with genomic islands acquired via horizontal gene transfer, provides the bacterium with the extraordinary metabolic flexibility required to adapt to a wide variety of environmental stresses.

The practical significance of the strain lies primarily in its efficient degradation of xenobiotics, particularly thiocarbamate and triazine herbicides used in agriculture practice. In the presence of herbicides, the production of specific enzymes—such as ThcA aldehyde dehydrogenase, ThcE alcohol dehydrogenase and ThcF haloperoxidase—is induced in the bacterium. The degradation process is directly driven by an inducible cytochrome P450 monooxygenase module (*thcB*, *thcC*, *thcD*) located on a genomic island. Interestingly, its sequence is almost identical to one found on a 77 kb plasmid of *Rhodococcus* sp. TE1 isolated from a maize field in Canada, proving that global chemical exposure triggered similar evolutionary responses (horizontal gene transfer) in soil bacteria.

In addition to environmental remediation, the NI86/21 strain and its close relatives play a key role in biocontrol and mycotoxin decontamination. The bacterium effectively degrades mold toxins (that can contaminate agricultural crops), including aflatoxin B1, zearalenone, and T-2 toxin. In our work, we investigated the genetic background of the strain's AFB1-degrading ability.

Its diverse enzyme arsenal responsible for pesticide and toxin degradation makes this strain one of the most valuable subjects in modern agricultural biotechnology and food safety research.

Prognostic Value of Anaerobic Bacterial Genus in Anaerobic Bacteraemia

Renátó Kovács^{1,2}; Andrea Harmath^{1,3}, Ágnes Jakab^{1,2}, Zoltán Tóth^{1,2}, Aliz Bozó^{1,2}, Bence Balázs^{1,2}, László Majoros^{1,2}

¹Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; ²Medical Microbiology, Clinical Centre, University of Debrecen, Debrecen, Hungary; ³Doctoral School of Pharmaceutical Sciences, University of Debrecen, Debrecen, Hungary

Anaerobic bacteraemia represents an uncommon yet clinically significant bloodstream infection with considerable mortality. While host-related factors are established determinants of outcome, the independent prognostic impact of the causative anaerobic pathogen remains insufficiently characterized. This study aimed to identify predictors of mortality in anaerobic bacteraemia and to evaluate whether genus-level microbiological classification offers prognostic information beyond established clinical risk factors. A retrospective cohort study was performed, including 96 patients with monomicrobial anaerobic bacteraemia treated at a tertiary-care centre in Hungary between January 2021 and December 2025. Demographic, clinical, and microbiological characteristics were collected retrospectively. Thirty- and 180-day survival were assessed using Kaplan–Meier analysis. Predictors of mortality were evaluated by Cox proportional hazards regression, and nested multivariable models were compared to determine the incremental prognostic value of bacterial genus. The median patient age was 68 years. The most frequently identified genera included *Bacteroides* (33%), *Clostridium* (17%), *Actinomyces* (16%), and *Fusobacterium* (9%). All-cause mortality rates were 43.8% at 30 days and 54.2% at 180 days. Survival differed significantly among anaerobic genera at 180 days (log-rank $p=0.033$), with *Clostridium* spp. bacteraemia exhibiting the highest crude mortality (75.0%). In multivariable Cox regression, liver cirrhosis (HR 2.73, 95% CI 1.14–6.52; $p=0.024$), renal failure (HR 2.48, 95% CI 1.22–5.07; $p=0.012$), and *Clostridium* spp. bacteraemia (HR 3.18, 95% CI 1.41–7.17; $p=0.005$) were independently associated with mortality. Incorporating bacterial genus significantly improved model fit compared to clinical variables alone (likelihood-ratio $p=0.003$). The final model demonstrated strong discrimination, with AUC values of 0.84 and 0.80 for 30- and 180-day mortality, respectively. Anaerobic bacteraemia was associated with elevated short- and long-term mortality. Beyond host-related risk factors, bacterial genus offered independent prognostic information, with *Clostridium* spp. bacteraemia identifying a particularly high-risk patient subgroup. Genus-level microbiological identification may facilitate early clinical risk stratification in patients with anaerobic bloodstream infection.

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Early transcriptional response of *Candidozyma (Candida) auris* in the presence of *Pseudomonas aeruginosa*

Fruzsina Kovács¹, Aliz Bozó¹, Ágnes Jakab¹, Andrea Harmath¹, Ágota Ragyák², Zoltán Tóth¹, Bence Balázs¹, Noémi Balla¹, László Majoros¹, Renátó Kovács¹

¹University of Debrecen, Faculty of Medicine, Department of Medical Microbiology, Debrecen, Hungary, ²Department of Inorganic and Analytical Chemistry, Agilent Atomic Spectroscopy Partner Laboratory, University of Debrecen, Debrecen, Hungary

Bacterial–fungal polymicrobial infections are of increasing concern, particularly in healthcare-associated environments. *Candidozyma (Candida) auris* and *Pseudomonas aeruginosa* frequently coexist in clinical settings and form biofilms, where their interactions may influence microbial growth, virulence, and infection outcomes. The mechanisms underlying the early interactions between these pathogens remain poorly characterized. This study aimed to characterize the early response of *C. auris* during interaction with *P. aeruginosa*, focusing on fungal growth, extracellular glucose and metal availability, transcriptional adaptation, and virulence-related outcomes in vivo. In vitro growth analysis, extracellular glucose and metal quantification, RNA sequencing, and a murine polymicrobial infection model were employed. Within 2 hours of bacterial addition, *C. auris* cell counts increased from $3.3 \pm 0.6 \times 10^6$ to $7.1 \pm 0.8 \times 10^6$ CFU/mL in monoculture, but only reached $4.9 \pm 0.4 \times 10^6$ CFU/mL in co-culture. At this time point, bulk extracellular glucose concentrations were similar between *C. auris* monoculture and co-culture. Extracellular iron and zinc concentrations also did not differ significantly between the two conditions at 2 hours. More pronounced differences were observed at later time points, with extracellular iron concentrations approximately 49% lower in co-culture than in *C. auris* monoculture at 4 hours, and extracellular zinc concentrations approximately 49% lower at 6 hours. Transcriptomic analysis identified 210 upregulated and 124 downregulated genes, indicating extensive remodeling of pathways associated with translation, carbon metabolism, stress responses, lipid metabolism, and cell-surface organization. Notably, transcriptional changes in iron-homeostasis-associated genes were detectable before significant differences in bulk extracellular iron concentrations emerged. In vivo, polymicrobial infection resulted in an approximately 1.4-log₁₀ reduction in the *C. auris* kidney burden, while the *P. aeruginosa* burden was not significantly altered. In summary, early interaction with *P. aeruginosa* is associated with reduced *C. auris* proliferation and broad transcriptional remodeling that precedes detectable changes in extracellular iron and zinc availability. These findings support a multifactorial interaction involving early metabolic and stress adaptation, followed by changes in the extracellular environment, rather than an effect attributable to competition for a single nutrient.

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The role of plant-fungus interaction and host plant developmental stage in peptaibol production by *Trichoderma longibrachiatum* f. *bissettii*

Fanni Kovács^{1,2}, Gergő Terna^{1,2}, Henriett L. Hunkár^{1,2}, Ákos Rozsnyói^{1,2}, Dániel Hercegfalvi^{1,2}, Dóra Balázs¹, Chetna Tyagi¹, András Szekeres¹, Mónika Varga¹, Isidora Knežević³, Helena Todorović³, Jovan Lazarević³, Gordana Racić³, Csaba Vágvolgyi¹, Tamás Papp¹, László Kredics¹, Tamás Marik¹

¹ Department of Biotechnology and Microbiology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary

² Doctoral School of Biology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary

³ Tamiš Research and Development Institute, Novoseljanski put 33, 26000 Pančevo, Serbia

Trichoderma species are soil-borne filamentous fungi widely used for their beneficial effects on plants, including growth promotion, enhanced disease resistance, and improved stress tolerance. Several species produce peptaibols, non-ribosomally synthesized antimicrobial peptides whose membrane-active properties contribute to biocontrol activity and plant–microbe interactions. However, peptaibol production is highly variable and the factors regulating its production during interaction with living plants remain poorly understood.

This study aimed to determine how the presence and developmental stage of tomato (*Solanum lycopersicum*) plants affect peptaibol production by *Trichoderma longibrachiatum* f. *bissettii* SZMC 12546, and whether the observed response is associated with active plant–fungus interaction. Tomato plants were cultivated on Murashige and Skoog (MS) medium, and the fungus was inoculated at four developmental stages: sowing, germination, cotyledon stage, and first true leaf stage. To distinguish the effect of living plants from residual plant-derived compounds, additional treatments included inoculation onto MS medium after plant removal and plant-free controls. Malt extract agar (MEA) served as a high-production reference.

Co-cultivation with living tomato plants markedly increased peptaibol production in a developmental stage-dependent manner. Intensive sporulation was observed in the rhizosphere of living plants, whereas this response was absent on plant-free MS medium. Peptaibols were extracted using chloroform:methanol (2:1, v/v) and quantified by agar diffusion bioassay against *Micrococcus luteus*. Production on MS medium was significantly lower than on MEA, while co-cultivation with tomato plants substantially enhanced peptaibol levels. The highest production was observed when the fungus was introduced at the first true leaf stage, reaching levels comparable to those measured on MEA. No bioactive compounds were detected in extracts of tomato plants cultivated without the fungus.

These findings demonstrate that peptaibol biosynthesis by *T. longibrachiatum* f. *bissettii* is strongly influenced by plant–fungus interaction and modulated by the developmental stage of the host plant. The stage-dependent response suggests that plant developmental status may affect fungal secondary metabolism and provides a basis for investigating plant-derived signals involved in peptaibol induction. These results contribute to a better understanding of *Trichoderma*–plant interactions and may support the optimization of peptaibol production for future biocontrol applications. Detailed peptaibol profiling by HPLC–MS is underway to further characterize the metabolite composition.

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Stress tolerance and antagonistic potential of rhizosphere-derived microorganisms

László Kredics¹, Emese Judák¹, Henrietta Allaga¹, András Varga¹, Ingrid Melinda Gyalai², Flórián Kovács², Csaba Vágvölgyi¹, Orsolya Kedves¹

¹ Department of Biotechnology and Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

² Institute of Plant Sciences and Environmental Protection, Faculty of Agriculture, University of Szeged, Hódmezővásárhely, Hungary

The successful application of beneficial microorganisms in agriculture requires not only the expression of plant growth-promoting traits but also the ability to remain active under variable environmental conditions. Microorganisms introduced into the rhizosphere may encounter fluctuations in temperature, pH and water availability, while interactions with plant-associated pathogens can further influence their performance. Therefore, the combined assessment of environmental stress tolerance and antagonistic activity represents an important step in the functional characterization of rhizosphere-derived microorganisms.

Culturable microorganisms were isolated from the rhizosphere of cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), pepper (*Capsicum annuum*) tomato (*Solanum lycopersicum*), and wheat (*Triticum aestivum*). Selection of twenty bacterial isolates from each plant species resulted in a culture collection of 100 bacterial strains. In addition, selective media were also used to recover 20–25 *Trichoderma* isolates.

The isolate collection is evaluated for tolerance to environmental factors relevant to survival and activity in plant-associated habitats, including different temperatures, pH conditions and levels of water availability. In parallel, selected bacterial and fungal isolates are tested for antagonistic activity against plant-pathogenic fungi. The combined analysis of stress-response patterns and antagonistic potential enables the comparison of isolates originating from different crop rhizospheres and supports the identification of microorganisms with robust and agriculturally relevant functional characteristics.

This integrated approach provides a basis for selecting promising rhizosphere-derived microorganisms for subsequent plant-based experiments. Linking taxonomic identity with environmental tolerance and antagonistic activity may contribute to a better understanding of the functional diversity of culturable rhizosphere microbiota and support the development of sustainable microbial strategies aimed at improving plant resilience and biological protection in crop production.

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System-specific ecotoxicological responses to anatase and rutile TiO₂ nanoparticles in prokaryotic and eukaryotic models

Gergely Krett¹, Rózsa Farkas¹, Máté Varga², Tamás Annus², Linda Marzougui³, Ádám Solti³, Károly Bóka⁴ and Erika Tóth^{1,5}

¹ Department of Microbiology, Eötvös Loránd University, Budapest, Hungary ² Department of Genetics, Eötvös Loránd University, Budapest, Hungary ³ Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, Budapest, Hungary ⁴ Department of Plant Anatomy, Eötvös Loránd University, Budapest, Hungary ⁵ Research and Development Center, Eszterházy Károly Catholic University, Eger, Hungary

The use of nanoscale materials has increased rapidly in recent decades, with titanium dioxide (TiO₂) nanoparticles among the most widely produced nanomaterials. Their increasing release into the environment may pose ecological risks; however, their toxicity can strongly depend on particle properties and exposure conditions. In our study, we compared the effects of anatase and rutile TiO₂ nanoparticles in prokaryotic and eukaryotic systems, including aquatic bacterial communities, bacterial cultures, plant model systems (*Sinapis alba*, *Triticum aestivum*), the SOS Chromotest, and developmental assays using zebrafish (*Danio rerio*). Nano-TiO₂ exposure markedly altered the composition of freshwater bacterial communities, decreasing the relative abundance of certain taxa (Actinomycetota, *Flavobacterium*, *Limnohabitans*), while representatives of other genera (*Pseudomonas*, *Sediminibacterium*, *Haliscomenobacter*, *Hydrogenophaga*) became dominant. Rutile caused more pronounced changes in bacterial community composition and greater bacterial cell damage, whereas anatase showed stronger effects in the plant models, particularly affecting hypocotyl growth and plant water balance. The SOS Chromotest indicated limited acute genotoxicity, and under the tested conditions, TiO₂ nanoparticles did not significantly affect zebrafish survival or final larval body length; however, they delayed hatching at the highest concentration (1000 ppm). Overall, our results show that the toxicity of TiO₂ nanoparticles can vary considerably among biological systems and depends on the combined influence of particle properties and organism-specific structural and physiological characteristics.

Antibiotic-resistant coliform bacteria in Hungarian recreational water

Krisztina Kurucz-Lajtos¹, Ábel Csongor Németh¹, Boglárka Pollák¹, Balázs Seres¹, Márta Vargha^{1,2}, Bernadett Khayer^{1,3}

¹ National Center for Public Health and Pharmacy, Department of Methodology and Public Health Laboratories, Division of Environmental Health Laboratories, Budapest, Hungary

² University of Debrecen, Faculty of Health Sciences, One Health Institute, Department of Planetary Health, Debrecen, Hungary

³ University of Debrecen, Faculty of Health Sciences, One Health Institute, Department of Infection Control and Hospital Epidemiology, Debrecen, Hungary

This study is connected to the UrbanBlueHealth project, which focuses on the water quality and safety of natural bathing waters. The proliferation of indigenous bacteria and the emergence of new pathogens, mainly due to climate change, can also endanger bathing safety. The discharge of treated and untreated wastewater, as well as polluted watercourses also poses a risk.

This work focused on the isolation of antibiotic-resistant coliform bacteria and investigating their resistance. Water samples were collected from three locations during the 2025 bathing season. These were the Danubian Postás Beach in Szentendre (an identified bathing water), and two sources of pollution identified as contributing to the site: the Bükkös stream and the area of the combined sewage overflow outlet. During sample processing, several culture-based methods were used in parallel to determine the number of normal and ESBL-resistant coliform bacteria. To this end, the culture media were supplemented with 10 mg/L of the antibiotic ceftriaxone. Resistance types were also examined at strain and community levels.

In case of membrane filtration methods, evaluation was impossible due to the high number of colonies. Therefore, the sampling sites could primarily be compared based on the results of the MPN-based methods (Colilert-18 and MUG). The highest *E. coli* values were found in the Bükkös stream, while the lowest values were observed in the Postás Beach samples. A similar trend was observed among the results with ceftriaxone-supplemented media. A total of 51 coliform strains were successfully isolated and identified at species level by MALDI-TOF MS. The antibiotic susceptibility of the strains was examined using the MASTDISC disk sets. With the D72C set, ESBL positivity was the most common result (26 strains), although AmpC-positive and combined AmpC and ESBL phenotypes were also observed. Using D73C disk set, most strains produce “equivocal” or “negative” results, but KPC-positive, OXA-48-positive, MBL-positive, and AmpC + porin loss phenotypes were also detected. Based on these results, ESBL- and AmpC-like phenotypes were dominant among the examined strains, while carbapenemase-related phenotypes were found only in smaller numbers. During the community qPCR analysis, the presence and quantity of five resistance genes were determined in the water samples. The CTX-M-1, CTX-M-9, and OXA-48-like genes occurred most often and at the highest concentrations, mainly in the Bükkös stream samples. In contrast, the KPC and NDM genes were present only in the Danube, during a flooding period.

Overall, the results suggest that antibiotic-resistant bacteria and resistance genes may both be present in the examined surface waters, therefore their monitoring may also be important from a public health perspective. This work is supported by the Interreg CE0200763 UrbanBlueHealth project.

EICOSAPENTAENOIC ACID PRODUCTION BY *PHAEODACTYLUM TRICORNUTUM* UNDER DIFFERENT CULTURE CONDITION AND THE ALGAL EXTRACT EFFECTS ON MITOCHONDRIAL DYSFUNCTION

B. Kutasi¹, A. Kolonics², V. Léner³, P. Futó¹, M. Futó¹, *J. Kutasi¹

¹Albitech Biotechnology Ltd., 1045 Budapest, Park of Research Berlini, Berlini st. 47-49., 5/A. Hungary; ²University of Physical Education, Budapest, Hungary; ³Dept of Experimental Pharmacology, National Institute of Oncology, Budapest, Hungary

P. tricornutum is a marine microalga, which is rich in bioactive molecules known to be protective against oxidative stress, such as n-3 long-chain polyunsaturated fatty acids and fucoxanthin [PMID: 32575640]. We have isolated the diatom *Phaeodactylum tricornutum* from those parts of the Norwegian fjords where glacial meltwater mixes with salt water (brackish zones). We examined these *P. tricornutum* microalgal strain cultures under various cultivation conditions to assess their ability to produce eicosapentaenoic acid (EPA) – the most common polyunsaturated fatty acid (PUFA). In our experiments, we investigated how sodium chloride, the nitrogen source, phosphate, the initial pH, and the CO₂ content of the culture medium affect the amount of eicosapentaenoic acid (EPA-P) phospholipid derivative produced by *P. tricornutum*. The EPA-P content of the biomass was increased by a low pH of the culture medium, higher concentrations of vitamin B12 and nitrate, and a lower concentration of sodium chloride. EPA- is highly likely to be bound to polar (membrane) lipids, and its role is manifested in the membrane permeability of microalgae. The synthesis of phospholipids is expected to increase the EPA-P content of the cells. Promising benefits of six-month *P. tricornutum* microalgae supplementation on cognitive function and inflammation in healthy older adults with age-associated memory impairment was proved [1], however its effect on cellular model of Parkinson's disease was not studied. Parkinson's disease (PD), a common neurodegenerative disease is characterized by the progressive loss of dopaminergic neurons in the substantia nigra. PD is an age related neurodegenerative disorder believed to originate in part via reactive oxygen species overproduction, leading to oxidative stress and mitochondrial dysfunction [2]. Paraquat (PQ²⁺), harms human cells mainly through redox cycling, which creates massive amounts of toxic reactive oxygen species (ROS) and leads to severe oxidative stress, mitochondrial damage, and apoptosis [3]. The effect of water resolved *P. tricornutum* CO₂ freeze-dried extract was examined on PQ²⁺ induced cellular toxicity by MTT assay in SH-SY5Y cells. Parallel cellular ATP production was measured by bioluminescent luciferase assay (FLAAM). *P. tricornutum* treatment mildly increased the viability of PQ²⁺ exposed SH-SY5Y cells and elevated cellular ATP levels in ADP coupled state 3 of the mitochondria. In conclusion, molecular species of *P. tricornutum* may exhibit mild neuroprotection against PQ²⁺-induced neurotoxicity that can be therapeutically beneficial in Parkinson's disease as an add-on therapy but more specific models are needed to validate its efficacy.

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Structural Asymmetry and MIC Profiles of Peptaibiotics in Different *Trichoderma* Strains

Henriett L. Hunkár^{1,2}, Fanni Kovács^{1,2}, Dóra Balázs¹, Gergő Terna^{1,2}, Ákos Rozsnyói^{1,2}, András Szekeres¹, Mónika Varga¹, Isidora Knežević³, Helena Todorović³, Jovan Lazarević³, Gordana Racić³, Csaba Vágvolgyi¹, Tamás Papp¹, László Kredics¹, Chetna Tyagi¹, Tamás Marik¹

¹ Department of Biotechnology and Microbiology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52, H-6726, Szeged, Hungary ² Doctoral School of Biology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52, H-6726, Szeged, Hungary ³ Tamiš Research and Development Institute, Novoseljanski put 33, 26000 Pančevo, Serbia

Trichoderma species produce peptaibiotics, which comprise two major groups: peptaibols and lipopeptaibols. Peptaibols are non-ribosomally synthesized peptides characterized by the presence of α -aminoisobutyric acid, an N-terminal acetyl group, and a C-terminal amino alcohol. Lipopeptaibols additionally possess an N-terminal lipid or fatty acid moiety. Although the biological activity of both peptide groups is mainly associated with membrane disruption, several species produce exclusively peptaibols. However, no known species produces only lipopeptaibols, which always co-occur with standard peptaibols. This study investigates why specific fungal lineages possess the machinery for exclusive peptaibol production, while no species dedicates its biosynthetic pathways solely to lipopeptaibol biosynthesis.

The aim of this study was to isolate and characterize peptaibols and lipopeptaibols produced by selected *Trichoderma* strains, to investigate their structural and physicochemical properties, perform molecular dynamics simulations, and compare their biological activities. The 9 investigated strains showed considerable variation in their peptaibiotics profiles. *T. atroviride* SZMC 28748 produced long, 19-residue peptaibols with exclusively Gln termini. *T. cf. dorotheopsis* SZMC 28390 produced mainly long peptaibols, while 16-, 17-, and 18-amino-acid variants were also detected, probably as the result of module skipping. While both examined *T. cf. strigosellum* strains produce short lipopeptaibols and long peptaibols, SZMC 28391 yields exclusively Gln-containing sequences at their C-terminal, whereas SZMC 28007 yields both Gln- and Glu-containing sequences. *T. koningii* SZMC 28387 produced short and medium lipopeptaibols together with long peptaibols. *T. cf. dorotheopsis* SZMC 28005 produced medium and long lipopeptaibols as well as long peptaibols. *Trichoderma hamatum* SZMC 28747 produced short, medium and long lipopeptaibols together with long peptaibols. Both *T. helicum* strains produced short lipopeptaibols, short peptaibols, and long peptaibols, however, strain SZMC 27992 includes both Gln and Glu, while SZMC 28389 contains only Gln in its long peptaibols.

The selected strains were cultivated on a large scale, and crude extracts were prepared from both the fungal biomass and the culture medium. Agar diffusion assays against *Micrococcus luteus* revealed marked differences in the biological activity of peptaibiotics accumulated in the biomass and the culture medium. This was particularly evident for *T. cf. strigosellum* SZMC 28391 and SZMC 28007. The peptaibols produced by these strains differ at a single structural position preceding the C-terminal amino alcohol: strain SZMC 28391 contains only Gln, whereas strain SZMC 28007 contains predominantly Gln together with a smaller proportion of Glu. Furthermore, crude extracts containing lipopeptaibols exhibited remarkably higher bioactivity compared to those containing peptaibols exclusively. Biological activities of individual fractions will be evaluated, and their antibacterial activities compared via the determination of minimum inhibitory concentrations. Molecular dynamics simulations will complement these experiments detailing structural behaviour. Together, these approaches will clarify the relationships between peptaibiotic structure and biological activity, supporting the future development of naturally derived antimicrobial compounds.

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Context-Dependent and Temporal Impact of AMF Inoculation of Tomato Under Dual Biotrophic Pathogen Infection

Lívía László¹; Beatrix Pethőné Rétháti¹; Katalin Salánki²; Asztéria Almási²; Alexandra Pintye²; Katalin Posta¹

¹ Hungarian University of Agriculture and Life Sciences, Institute of Genetics and Biotechnology, Department of Microbiology and Applied Biotechnology, 2100 Gödöllő, Hungary

² HUN-REN Centre for Agricultural Research, Plant Protection Institute, Department of Plant Pathology, 1116 Budapest, Hungary

Arbuscular mycorrhizal fungi (AMF) are important drivers of plant resilience, well-documented for mitigating abiotic stresses such as drought, salinity, and heavy metal toxicity by augmenting nutrient and water acquisition and priming host defence networks. However, the capacity of AMF symbioses to modulate host responses during challenging single and combined infections with obligate biotrophic pathogens remains under-explored. Both *Erysiphe hortensiae* (the causal agent of tomato powdery mildew) and cucumber mosaic virus (CMV) pose severe global threats to greenhouse and field-grown crops, frequently triggering substantial yield losses. Management of these pathogens is inherently constrained by their biotrophic lifestyles, the scarcity of selective chemical controls, and the widespread emergence of fungicide resistance, emphasising the need for sustainable, biologically based plant protection strategies.

This study investigates how AMF colonisation alters the physiological and defensive responses of tomato (*Solanum lycopersicum* L. cv. 'Moneymaker') subjected to single and dual challenge by *E. neolycopersici* and CMV. Plants were cultivated under controlled greenhouse conditions and divided into two groups: an AMF-inoculated treatment (AMF+) receiving an inoculum mixture of *Rhizophagus clarus* and *Rhizophagus intraradices*, and a non-inoculated control group (AMF-). Following a 5.5-week mycorrhizal establishment phase, AMF+ and AMF- plants were exposed to *E. hortensiae*, CMV, or their combination to simulate biotic stress. Host physiological parameters, growth indices, and level of infection were quantified post-inoculation.

Our preliminary results indicate that AMF-mediated effects are dynamic and context-dependent. The outcome of mycorrhizal symbiosis appears to vary depending on the specific pathogen combination, with trends suggesting that the impact of AMF may undergo temporal shifts over the course of infection. These findings underscore the complex interactions between symbiotic fungi, viral and fungal biotrophic pathogens, and host defence signalling. Unravelling these time-dependent and pathogen-specific dynamics provides valuable insights into mycorrhizal-induced stress response, highlighting both the opportunities and nuances of integrating AMF into sustainable crop protection strategies.

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Increasing number of carbapenem-resistant *Bacteroides* strains in Hungary and an outlook to their plasmid biology

Andrea Lázár¹, László Orosz¹, Mariann Ábrók¹, Ágnes Sarkadi-Nagy¹, Emese Juhász², Dóra Neubrandt³, Bence Tarnóczy¹, Danhui Heo¹, Katalin Kristóf², Erzsébet Nagy¹, Katalin Burián¹, József Sóki¹

¹ Institute of Medical Microbiology, Albert Szent-Györgyi School of Medicine and Health Centre, University of Szeged, Hungary,

² Institute of Laboratory Medicine, Semmelweis University, Budapest, Hungary ³SYNLAB Hungary Ltd., Budapest, Hungary

The paramount importance of the normal microbiota in several physiologic processes is well-explored today, including to be a reservoir for antibiotic resistance genes and multidrug-resistant bacteria. *Bacteroides* are thought to contribute to these processes profoundly. In addition, they show low resistance rates to the wide spectrum carbapenems but carbapenem resistance can occur among them and aggravate therapy. The best-known resistance mechanisms against carbapenems among *Bacteroides* strains are the species-specific *cfiA* (*B. fragilis*) and *crxA* (*B. xylanisolvens*) genes activated by insertion sequence (IS) elements giving rise ca. 1 % resistance rates. Another interesting feature of them is that *Bacteroides* can harbour in high percentage small molecular weight cryptic plasmids. In this report we summarize our recent results on appearing of highly carbapenem-resistant *B. fragilis* strains in Hungary and have an outlook on the spread of the above mobile genetic elements (MGEs) that may make these processes more intriguing.

Of 341 *B. fragilis* and 10 *B. xylanisolvens* recently isolated strains 6 (1.2%) and 1 (10%) resistant isolate were included in further characterization. These numbers meant an increasing prevalence since only rare sporadic cases were found previously in Hungary so far. Six *B. fragilis* strains were *cfiA*-positive and four of them were IS element-positive with varying activating IS types (IS1169, IS612, IS1380-like). The *B. xylanisolvens* strain was *crxA*-positive without IS element activation. Other antibiotics, including metronidazole, Etests results and the analysis of genomes showed that the strains were not multidrug-resistant. Additionally, the evolution of cryptic plasmids among Hungarian *Bacteroides* strains and an emphasis of them in virulence and antibiotic resistance in global terms are also discussed.

We can expect a deteriorating carbapenem resistance situation among *Bacteroides* strains in Hungary and the facilitation of the processes involving them in the gut microbiome by MGEs is warning as well.

Genomics-referenced typing of vancomycin-resistant *Enterococcus faecium* isolates using MALDI-TOF mass spectrometry and Raman spectroscopy

Anna Lorencz¹, Olivér Mohai¹, Katalin Burián¹, Gábor Katona², László Orosz¹

¹Institute of Medical Microbiology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary

²University of Szeged, Faculty of Pharmacy, Institute of Pharmaceutical Technology and Regulatory Affairs

Rapid discrimination of vancomycin-resistant *Enterococcus faecium* (VREfm) isolates is important for detecting transmission in healthcare settings. This study evaluated whether MALDI-TOF mass spectrometry and Raman spectroscopy can provide phenotypic discrimination of genomically defined VREfm lineages. MALDI-TOF and Raman spectra were analysed for 13 clinical VREfm isolates previously characterized by core-genome multilocus sequence typing (cgMLST) and multilocus sequence typing (MLST) at the National Center for Public Health and Pharmacy. Nine isolates belonged to the ST117 clade, two to ST80 and one to ST18, while one isolate was a single-locus variant of ST117. MALDI-TOF spectra of ST117-related isolates showed characteristic differences around 6407 Da and within the 6.9–7.2 kDa region. Hierarchical clustering revealed partial grouping of ST117 isolates but also overlap with other sequence types, suggesting that discrimination depended on combined spectral patterns rather than on a single peak. Principal component analysis and hierarchical clustering of Raman spectra distinguished the ST117, ST80 and ST18 isolates. However, the cgMLST types within ST117 showed substantial spectral overlap. These findings indicate that MALDI-TOF mass spectrometry and Raman spectroscopy may provide rapid, genomics-complementary phenotypic typing of VREfm lineages at the MLST level. Further validation using larger isolate collections is required to determine whether closely related cgMLST variants can be reliably discriminated.

A comparison of lignocellulose-degrading enzyme activity of *Pleurotus ostreatus* strains cultivated in winter and summer

Csaba László Maller¹, Lóránt Hatvani², Adrienn Nagy², Balázs Vajna¹

¹ Department of Microbiology, Eötvös Loránd University, Pázmány Péter sétány 1/C, 1117 Budapest, Hungary,

² Pilze-Nagy Ltd., Talfája tanya 47/B, 6000 Kecskemét, Hungary

Pleurotus ostreatus is an easily cultivable white-rot fungus that naturally grows on the surface of trees. White-rot fungi are capable of degrading lignocellulose, which contributes to their outstanding biodegradation potential. *Pleurotus ostreatus* is no exception and is also one of the most widely cultivated edible mushroom species complex at an industrial scale. The broader aim of our research is to exploit the biodegradation capabilities of *P. ostreatus* for the removal of soil pollutants. Therefore, we consider it important to obtain as much information as possible on the activity of the lignocellulose-degrading enzymes of the mushroom, including laccase and manganese peroxidase, as well as the changes in their activity throughout the full cultivation cycle.

In this study, we investigated the effects of environmental differences associated with the winter and summer seasons on the enzyme activities of several commercially used *P. ostreatus* strains during a complete cultivation cycle. The strains included the widely used HK35, the heat-tolerant Etna, and the cold-tolerant Kryos strain. Samples were collected at three time points during the cultivation cycle, which had been established in previous experiments: (1) 7–10 days after inoculation with mushroom spawn, (2) during the first flush (days 28–35), (3) and at the end of the cultivation cycle (days 45–50). Following each sampling, the activities of six different enzymes were determined: laccase, manganese peroxidase, endocellulase, endoxylanase, β -glucosidase, and exochitinase. In addition, fungal growth dynamics were investigated using qPCR.

Our results indicate that seasonal variation and the associated differences in environmental conditions do not significantly affect the activities of the lignocellulose-degrading enzymes. No significant differences in enzyme activity were observed among the three strains. The activity of laccases, the most important group of lignin-degrading enzymes in *P. ostreatus*, remained stable throughout the cultivation cycle and, contrary to our previous observations, did not decrease significantly by the end of the cycle. Manganese peroxidase activity increased towards the end of the cultivation cycle, as expected. The qPCR measurements are currently in progress, and the results will be presented during the conference.

Our findings are promising about the potential biodegradation capacity of *P. ostreatus* strains, as inferred from their enzyme activities, suggesting that it is not substantially affected by seasonal conditions or by the commercially available strains tested. These results indicate that, in future experiments and potentially in industrial applications, the choice of fungal substrate may not need to be restricted based on either cultivation season or strain.

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Enhanced citric acid fermentation by *Aspergillus niger* via transporter manipulation, Part A: Characterization and performance of mutants at laboratory scale

Alexandra Márton¹, Josee Vandal², Vivien Bíró³, Erzsébet Fekete¹, Erzsébet Sándor⁴, Adrian Tsang², Levente Karaffa¹

¹ Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary ², Centre for Structural and Functional Genomics, Concordia University, Montreal, QC, Canada, ³ Department of Molecular Biotechnology and Microbiology, University of Debrecen, Debrecen, Hungary, ⁴ Institute of Plant Protection, University of Debrecen, Debrecen, Hungary

Citric acid is the leading industrial organic acid, with an annual global production exceeding 2.5 million tons for the food, pharmaceutical, and cosmetic industries. Manufacturing relies on submerged fermentation using the fungus *Aspergillus niger*, a robust platform favored for its high secretion capacity, low pH tolerance, cheap substrate utilization, and proven safety. High-yield citrate fermentation requires high sugar (>120 g/L), low pH (<1.5), high oxygen, and strict nutrient limitation. Crucially, Mn²⁺ must not exceed 5 µg/L, as manganese acts as a metabolic switch toggling *A. niger* from citrate overflow to biomass growth. At the molecular level, cellular manganese uptake is mediated by the high-affinity transporter DmtA, while citrate export is driven by the plasma membrane exporter CexA, a major production bottleneck. Elevated manganese suppresses *cexA* transcription, directly linking manganese homeostasis to reduced citrate export.

To overcome this bottleneck, this study characterizes a novel double-transporter mutant combining *dmtA* deletion with *glaA*-driven *cexA* overexpression, evaluated against parental (NRRL2270) and single-mutant strains at laboratory scale. Under manganese deficiency, all strains achieved molar citrate yields above 80%, but the double mutant significantly outperformed the others, reaching 90%. It exhibited accelerated citrate accumulation (0.74 g/L/h) and a growth-decoupled, biphasic kinetic profile. Unlike traditional strains that continuously synthesize biomass, the double mutant completed biomass growth within 100 hours and shifted exclusively to organic acid excretion. This metabolic shift is driven by active CexA-mediated efflux, which depletes intracellular citrate, relieves feedback inhibition, and channels carbon flux directly into production. When subjected to high manganese levels (100 µg/L), which typically trigger severe yield loss and massive biomass growth (46 g/L) in the parental strain, the double mutant maintained an efficient overflow metabolism. It suppressed final biomass to just 9.1 g/L and compressed the fermentation period from 300 to 250 hours. This superior performance was powered by rapid carbon uptake, with a maximal glucose consumption rate of 1.61 g/L/h, outcompeting the single *cexA*^{OE} mutant by 178%. By decoupling growth from synthesis and eliminating the strict requirement for manganese purification at the laboratory scale, this dual-transporter engineering strategy establishes a productive, energy-efficient foundation for industrial citric acid manufacturing.

Enhanced citric acid fermentation by *Aspergillus niger* via transporter manipulation, Part B: Testing robustness and scalability at pilot scale

Alexandra Márton¹, Josee Vandal², Erzsébet Fekete¹, Erzsébet Sándor³, Adrian Tsang², Levente Karaffa¹

¹ Department of Biochemical Engineering, University of Debrecen, Debrecen, Hungary ², Centre for Structural and Functional Genomics, Concordia University, Montreal, QC, Canada, ³ Institute of Plant Protection, University of Debrecen, Debrecen, Hungary

Industrial citric acid manufacturing via submerged *Aspergillus niger* fermentation faces techno-economic challenges due to its extreme manganese sensitivity. Standard production requires strict manganese limitation (<5 µg/L), necessitating costly medium-purification methods like cation exchange or chemical precipitation. Furthermore, the hyper-acidic conditions (pH <2) required for citric acid overflow pose a corrosion risk in large-scale stainless-steel bioreactors. Since these alloys contain manganese, metal leaching from the vessel walls may disrupt overflow metabolism and reduces yields. This study evaluates the technological robustness and scalability of the $\Delta dmtA/cexA^{OE}$ double-transporter *A. niger* mutant engineered to maintain high-yield citrate production without prior manganese depletion.

To simulate industrial conditions, the double mutant was cultivated using untreated, non-ion-exchanged media with high native manganese levels (148–165 µg/L) and tested in a 15-L stainless-steel fermenter. Despite the elevated background manganese and hardware leaching, the engineered strain demonstrated resilience, achieving molar citric acid yields identical to those obtained in glass laboratory bioreactors. This robust performance eliminates the need for expensive medium treatment or electrochemical wall polishing. Morphological analysis revealed that this tolerance is rooted in the *dmtA* deletion. While high manganese triggers dispersed filamentous growth – which increases broth viscosity and impairs mass transfer – the double mutant maintained a production-optimized, pellet-dominated morphology. This architecture is dictated by low intracellular manganese levels regulated by DmtA, ensuring favorable rheological properties.

Scalability was validated by cultivating the strain in a 150-L pilot-scale bioreactor, keeping the initial impeller tip speed constant and maintaining dissolved oxygen at 30%. Although the pilot-scale environment introduced novel stressors, including increased hydrostatic pressure and a 0.4 bar overpressure, the double mutant sustained its growth-decoupled, biphasic kinetic profile. Rapid biomass formation was followed by intensive citric acid accumulation, resulting in a specific molar yield of 0.91. Remarkably, the fermentation period was compressed to just 192 hours for complete glucose exhaustion. By proofing process robustness against substrate impurities and hardware leaching, alongside accelerating production at an order-of-magnitude higher scale, this dual-transporter cell factory provides an economically viable framework for industrial citric acid manufacturing.

Virological screening of avian samples: special focus on adeno- and circoviruses

Lilla Katalin Máté¹, Balázs Harrach¹, Tamás Bakonyi², Herbert Weissenböck³, Krisztina Ursu⁴, Győző László Kaján¹

¹ HUN-REN Veterinary Medical Research Institute, Budapest, Hungary, ² University of Veterinary Medicine, Budapest, Hungary,

³ Pathology, Centre of Pathobiology, Department of Biological Sciences and Pathobiology, Vienna, Austria, ⁴ Eurofins Vetcontrol Ltd., Budapest, Hungary

Deforestation, intensive agricultural practices, urbanisation, live-animal trade, and the international movement of animals increasingly alter interactions among wildlife, domestic animals, and humans, creating new opportunities for pathogen transmission and host switching. The growing popularity of exotic birds as companion animals may further increase these risks. Despite their potential ecological, veterinary, and economic importance, our knowledge of the diversity, origin, evolution, and pathogenic potential of avian viruses remains limited. Mapping the avian virosphere is therefore essential for improving our understanding of viral emergence, supporting risk assessment and disease surveillance, and ultimately contributing to the conservation of wild bird biodiversity.

The present work focused primarily on members of the families *Adenoviridae* and *Circoviridae*, as viruses belonging to both groups have previously been detected in numerous domestic and wild bird species. The aim was to screen avian samples submitted for diagnostic examination for the presence of adenoviruses and circoviruses using previously established broad-range PCR assays with degenerate primers. Following virus detection, PCR products were subjected to Sanger sequencing to obtain preliminary information on the detected viruses. In the case of a strain representing a novel viral species, complete genome sequencing was subsequently performed using next-generation sequencing technology. The detected adenoviruses and circoviruses were compared with previously described viral types and strains by phylogenetic analysis, and the relationships among them were visualised using phylogenetic trees.

In the PCR screening, twenty-six samples (28%) yielded positive results for adenoviruses. Strains were classified in the genera *Aviadenovirus*, *Barthadenovirus* and *Siadenovirus*. Based on the sequence identity of the partial DNA polymerase amino acid sequence, twelve strains exhibited more than a 15% phylogenetic distance compared to members of previously recognised species, thus they may be eventually candidates for novel species. Also, we succeeded in identifying one complete genome belonging to the genus *Aviadenovirus* from a fieldfare (*Turdus pilaris*). In the circovirus screening, one sample (1%) from a chicken yielded a positive result; its sequence exhibited 60% amino acid identity to a Eurasian scops owl (*Otus scops*) CRESS-DNA virus. Its complete genome sequencing was unsuccessful.

Our findings contribute to the ongoing characterization of the avian virosphere and may expand current knowledge of the genetic diversity and genomic architecture of avian adenoviruses and circoviruses. Such investigations are essential for identifying previously unrecognized viruses and improving our understanding of their evolution, host associations, and potential for emergence in new host species.

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Generation and comparative characterization of NFAP2-, amphotericin B- and anidulafungin-resistant *Candidozyma auris* strains

Richárd Merber^{1,2}, Krisztián Laczi¹, Gábor Bende^{1,2,3}, Attila Farkas⁴, Gergely Maróti⁴, Rita Sinka⁵, Szabolcs Sáringer⁶, László Orosz⁷, Gábor Katona⁸, László Galgóczy¹

¹ Department of Biotechnology and Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary, ² Doctoral School of Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary, ³ Department of Theoretical Health Sciences and Health Management, Faculty of Health Sciences and Social Studies, University of Szeged, Szeged, Hungary, ⁴ Institute of Plant Biology, HUN-REN Biological Research Centre, Szeged, Hungary, ⁵ Department of Genetics, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary, ⁶ Department of Physiology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary, ⁷ Department of Medical Microbiology, Albert Szent-Györgyi Health Center, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary, ⁸ Institute of Pharmaceutical Technology and Regulatory Affairs, Faculty of Pharmacy, University of Szeged, Szeged, Hungary

Candidozyma (Candida) auris is an emerging multidrug-resistant human pathogenic yeast requiring novel therapeutic approaches. *Neosartorya (Aspergillus) fischeri* antifungal protein 2 (NFAP2) has previously been identified as a promising candidate. This study assessed the potential for resistance development in *C. auris* against NFAP2 compared to amphotericin B (AMB) and anidulafungin (ANI). Six independent lineages were evolved for NFAP2, AMB, and ANI in low-cationic medium (LCM), alongside six passaged control lineages (P), all derived from *C. auris* NCPF 8971 (WT). Only two of six parallel cultures adapted to 8× MIC of NFAP2, and one of six to 8× MIC of ANI. In contrast, all six parallels adapted to 32× MIC of AMB. Antifungal susceptibility testing revealed key cross-reactivities: NFAP2- and ANI-resistant strains exhibited reduced susceptibility to AMB; AMB-resistant strains displayed elevated tolerance to ANI; and ANI-resistant strains showed increased susceptibility to NFAP2. Representative strains were selected for further characterisation. Pseudohyphal growth assays demonstrated that NFAP2-resistant strains formed elongated cells rather than pseudohyphae. Scanning electron microscopy (SEM) indicated that the NFAP2-resistant biofilms did not contain pseudohyphal cells either, and that the extracellular matrix was decreased or absent. SEM also found that NFAP2-resistant strains experienced less pronounced morphological damage upon NFAP2 treatment. Surface-enhanced Raman spectroscopy revealed alterations in cell-wall components and lipoproteins in NFAP2-resistant strains, changes in cell-wall and lipid composition in AMB-resistant strains, and alterations in carbohydrate and protein scaffolds in ANI-resistant strains. Metabolic fitness and abiotic stress tolerance assays (cell wall, membrane, thermal, and UV stressors) revealed distinct fitness costs, with an AMB-resistant strain (AMB1) showing the most severe growth impairment. In *Galleria mellonella* infection models, one NFAP2-resistant strain (NFAP2/4/3) displayed significantly increased virulence compared to the WT, while other NFAP2-resistant strains maintained WT-like virulence. Conversely, all evaluated AMB, ANI, and P strains exhibited significantly reduced virulence. Illumina whole-genome sequencing against a PacBio-generated reference genome identified 16 nonsynonymous mutations that likely account for the observed changes in phenotype, structure and virulence. Overall, these findings demonstrate that *C. auris* has a similar propensity to develop high-level resistance to NFAP2 compared to ANI, but also causes elevated virulence. The present work of L.G. was financed by the Hungarian National Research, Development and Innovation Office - NKFIH, K 146131 project.

Biodegradation and Estrogenic Activity Assessment of Bisphenol A, 17 β -Estradiol, and Nonylphenol by Non-Pathogenic *Pseudomonas* Strains

Tamás Mireisz^{1,2}, Flóra Boglárka Horváth^{1,3}, Imre Boldizsár^{4,5}, Judit Háhn⁶, Edit Kaszab⁶, Erika Tóth^{1,2,3,7}

¹ Department of Microbiology, Eötvös Loránd University, Budapest, Pázmány Péter stny. 1/C, H-1117, Hungary ² Doctoral School of Environmental Sciences, Institute of Biology, Eötvös Loránd University, Budapest, Pázmány Péter stny. 1/C, H-1117, Hungary ³ Doctoral School of Biology, Institute of Biology, Eötvös Loránd University, Budapest, Pázmány Péter stny. 1/C, H-1117; Hungary ⁴ Department of Pharmacognosy, Semmelweis University, Üllői út 26, Budapest 1085, Hungary ⁵ Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Pázmány Péter sétány 1/C, Budapest 1117, Hungary ⁶ Department of Environmental Safety, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Páter Károly utca 1., Gödöllő, Hungary ⁷ Research and Development Center, Eszterházy Károly Catholic University, Leányka Str. 8/G, 3300 Eger, Hungary

Endocrine-disrupting compounds (EDCs), such as bisphenol A (BPA), 17 β -estradiol (E2), and nonylphenol (NP), are common in natural waters and can harm aquatic ecosystems.

This study evaluated the degradation capacity of non-pathogenic bacterial strains, selected from a collection of 98 bacterial strains isolated from Danube water in March 2023, toward BPA, E2, and NP, and assessed the estrogenic activity of these compounds and their biodegradation end-products.

During this research *Pseudomonas nicosulfuronedens* strain I12A and *Pseudomonas nitritireducens* strain I1B were studied for their degradation capacities: the bacterial strains were cultured in liquid medium containing the compounds at 1 mg/L for three weeks; degradation was quantified by Dionex Ultimate 3000 UHPLC coupled to an Orbitrap Q Exactive Focus mass spectrometer with electrospray ionization. Estrogenic activity and cytotoxicity of NP, BPA, E2 and their degradation residues were assessed with *Saccharomyces cerevisiae* reporter strains BLYES and BLYR (M. Eldridge and G. Saylor, University of Tennessee), respectively, via bioluminescence after 5 h incubation at 30 °C (Victor™ X Light 2030 luminometer).

According to the chemical analysis, I12A degraded BPA by 46.81%, E2 by 47.5%, and NP by 80%, while I1B achieved the highest removal, with BPA at 68.48%, E2 at 77.52%, and NP at 95.90%. These degradation results were consistent with the estrogenic activity assays: bioluminescence intensity decreased for both BPA and NP, reflecting their degradation. In contrast, no change in bioluminescence intensity was observed for E2, indicating that despite degradation of the 17 β -estradiol, its estrogenic activity persisted — likely due to the formation of estrogenic metabolites.

These findings show strong biodegradation and biotransformation potential of *P. nitritireducens* I1B and *P. nicosulfuronedens* I12A toward endocrine disruptors.

Further work at lower, environmentally relevant concentrations and broader screening of non-pathogenic bacteria are warranted for sustainable micropollutant bioremediation.

Molecular detection of Siberian sturgeon herpesvirus and Acipenser iridovirus-European from Russian sturgeon (*Acipenser gueldenstaedtii*) in Hungary

Gergő Mitró¹, Márton Hoitsy², Andor Doszpoly¹

¹ HUN-REN Veterinary Medical Research Institute, Budapest, Hungary, ² University of Veterinary Medicine, Department of Exotic Animal-, Wildlife-, Fish- and Honeybee Medicine, Budapest, Hungary

The most important viruses affecting sturgeon (Acipenseridae) farming include Siberian sturgeon herpesvirus (SbSHV) and Acipenser Iridovirus-European (AcIV-E). SbSHV is a double-stranded DNA herpesvirus belonging to the genus *Ictavirus* within the family *Alloherpesviridae*. AcIV-E is a large double-stranded DNA virus infecting sturgeons, which has been detected in several European sturgeon species and has been present in Hungary since 2023. Although previously classified among iridoviruses, whole-genome analysis resulted in the reclassification of AcIV-E into the family *Mimiviridae*. Both viruses may cause significant problems under intensive aquaculture conditions, with clinical signs including lethargy, anorexia, skin and gill lesions, petechial haemorrhages, and, in severe cases, high morbidity and mortality, potentially resulting in substantial economic losses.

Mucosal and organ samples from 41 sturgeons originating from both fish farms and natural water sources were examined for SbSHV and AcIV-E. The examined species included Russian sturgeon (*Acipenser gueldenstaedtii*), Siberian sturgeon (*A. baerii*), stellate sturgeon (*A. stellatus*), sterlet (*A. ruthenus*), and beluga (*Huso huso*). Viral detection was performed using real-time PCR targeting conserved regions of the DNA-dependent DNA polymerase gene for SbSHV and the major capsid protein (MCP) gene for AcIV-E. In five fish, viral loads of the following organs/tissues were investigated: mucosa, skin, fin, gill, and pooled internal organ samples.

13 fish tested positive for at least one virus. One fish was positive exclusively for AcHV-2, seven exclusively for AcIV-E, and five for both viruses. All positive samples were from farmed Russian sturgeon. AcIV-E was detected most reliably in gill and mucosal samples.

Our results demonstrate the presence of AcHV-2 and AcIV-E in Russian sturgeon farms in Hungary. As neither virus was detected in wild individuals, their introduction through stock replenishment is therefore considered likely. The comparable detectability of AcIV-E in mucosal and gill samples may facilitate future diagnostics. Further research is warranted to better characterise sturgeon viruses and prevent economic losses in aquaculture.

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Biocolors – Late-stage diversification of anthraquinone colorants with synthetic biology

István Molnár¹, Pradhuman Jetha¹, Natalia Maiorova¹, Esa Tanhola¹, Hannu Maaheimo¹, Tom Wirtanen¹, Dominik Mojzita¹, Chen Wang², Zhigang Zhou², Merja Penttilä¹

¹ VTT Technical Research Centre of Finland, Espoo, Finland; ² National Key Laboratory of Agricultural Microbiology, Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

Flavin-dependent halogenases (FDHs) are valuable catalysts for the late-stage diversification of small molecule scaffolds, modulating their biological activities and/or introducing orthogonal functionalities for regiospecific derivatization. We discovered two such enzymes (CkHAL1 and CsHAL2) from *Cortinarius* mushrooms that chlorinate anthraquinone skeletons from the emodin family. As opposed to the phenolic FDH CcRadH from the ascomycete *Chaetomium chiversii* that can modify only neutral anthraquinones such as emodin and questin, the substrate range of CkHAL1 and CsHAL2 also extends to acidic pigments such as endocrocin and dermolutein. Correspondingly, our data suggests that *Cortinarius* and ascomycete FDHs likely arose via parallel evolution. We also identified CsRedE, a *Cortinarius* flavin reductase (FRD) that efficiently supports CsHal2-mediated catalysis. The FDH-encoding genes are not clustered with those of the FRDs, nor with those of the polyketide synthases or *O*-methyltransferases for anthraquinone biosynthesis, indicating effective metabolic crosstalk. Furthermore, sequence alignments and docking studies identified critical amino acids and distinctive sequence elements that may govern the regioselectivity and substrate range of *Cortinarius* halogenases. The discovery of the FDH and FRD enzymes with substantial sequence divergence from those in Ascomycetes aids the identification of additional such enzymes from basidiomycete fungi, and their utilization for the synthetic biological production of halogenated natural and “unnatural” products in heterologous chassis. Meanwhile, selective C–H functionalization allows the tuning of the physicochemical and pharmacokinetic properties of anthraquinone pigments for industrial and pharmaceutical use, providing scalable and environmentally more responsible alternatives to chemical synthetic production or extraction of rare analogs from nature.

Rare Inflammatory Breast Disease in Women of Childbearing Age: *Corynebacterium kroppenstedtii* and *Corynebacterium bovis* associated Cystic Neutrophilic Granulomatous Mastitis via Two Case Reports

Mária Molnár¹, Noémi Anikó Bartha¹, Márta Csire¹, Judit Kovácsné Kalácska¹, Zsuzsanna Baranyák², Szilárd Domján², Attila Patócs¹

¹Department of Microbiology, Central Laboratory, National Institute of Oncology, Budapest, Hungary; ²Department of Breast and Soft Tissue Cancer Surgery, National Institute of Oncology, Budapest, Hungary

Cystic neutrophilic granulomatous mastitis (CNGM) is a rare, histologically distinct subtype of granulomatous mastitis that most commonly affects women of childbearing age. The presence of lipophilic *Corynebacterium* species is increasingly confirmed in the background of this condition. Because the clinical and radiological presentation can deceptively mimic malignancy, establishing an accurate diagnosis presents a multidisciplinary challenge.

We present the cases of two 39-year-old female patients who presented for evaluation due to unilateral breast pain, inflammation, and a palpable mass. Their symptoms did not respond to initial empirical antibiotic treatment. Primary imaging studies (ultrasound, mammography) initially described fibrotic tissue, skin thickening in the affected area, and subcutaneous edema. Due to morphological progression observed during follow-up imaging (US, MRI) and suspicion of malignancy, a tissue core biopsy was performed in both cases. Histopathological examination confirmed the pattern of CNGM, ruling out a malignant neoplastic process. Targeted microbiological sampling was performed at our institution: *Corynebacterium kroppenstedtii* was cultured from one patient, and *Corynebacterium bovis* from the other. Based on the antibiogram, targeted antibiotic therapy was initiated in both cases. The former patient was referred to an infectious disease clinic, while the latter—due to the development of a local abscess—was referred to the regionally competent surgical department for abscess incision and drainage, alongside continuing the already initiated antibiotic treatment.

Despite the growing number of case studies, the diagnosis of CNGM remains a major clinical challenge, and its treatment guidelines are highly heterogeneous. With our case presentation, we aim to draw attention to the fact that the underlying lipophilic *Corynebacterium* species are extremely fastidious; they cannot be isolated on standard culture media, or only following prolonged incubation (48–72 hours or longer). Consequently, microbiology laboratories may prematurely report the samples as negative or classify the findings as contamination (skin flora). To avoid diagnostic pitfalls, close communication between clinicians and microbiologists is essential. The cornerstone of effective therapy relies on the prolonged administration—often lasting for weeks or months—of lipophilic antibiotics with excellent tissue penetration (such as clarithromycin or doxycycline), as well as surgical incision and drainage when necessary.

Examination of the biocontrol potential of a *Microdochium majus* fungal isolate against the grapevine trunk disease pathogen *Botryosphaeria dothidea*

Nikolett Molnár^{1,2}, Ádám Novák¹, Adrienn Gomba-Tóth¹, Kálmán Zoltán Váczy¹, Zoltán Karácsony¹

¹Eszterházy Károly Catholic University, 3300 Eger, Eszterházy square 1

²Hungarian University of Agriculture and Life Sciences, 2100 Gödöllő, Páter Károly street 1

The fungal species *Microdochium majus* was previously known primarily as an important pathogen of monocotyledonous small-grain cereals, but it has recently been isolated for the first time from asymptomatic grapevine tissues as a potential endophyte. Previous studies demonstrated that this isolate (strain CBS 152328) can act as a necrotrophic mycoparasite against the grapevine grey rot pathogen *Botrytis cinerea*, suppressing its growth and preventing leaf necrosis. In the present study, we aimed to determine whether *M. majus* can act as an effective antagonist against another major pathogen infecting the woody tissues of grapevine, *Botryosphaeria dothidea* strain 99c1. To evaluate its biocontrol efficacy, we established *in vitro* dual cultures as well as *in vivo* inoculation experiments on grapevine cuttings. The active mycoparasitic behaviour of *M. majus* against *B. dothidea* strain 99c1 was clearly observed in the dual cultures. Examination of longitudinal sections of the inoculated cuttings revealed that the extent of wood necrosis was lower in the co-inoculated cuttings compared with the control plants infected solely with the pathogenic strain 99c1. Our results indicate that *M. majus* may provide protection not only against pathogens infecting green tissues, but can also serve as an effective antagonistic biocontrol agent against fungi causing wood diseases—such as *B. dothidea*—which we aim to confirm through further investigations.

Keywords: biocontrol agent, micoparasitism, grapevine trunk diseases

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Fusarium sporotrichioides: a novel casual agent of lily wilt

Orsolya Molnár¹, Ieuan Rhys Evans², László Palkovics^{3,4,5}, János Ágoston^{1,3}

¹ Department of Plant Pathology, Plant Protection Institute, HUN-REN Centre for Agricultural Research, Budapest, Hungary,

² Provincial Plant Pathologist, Food and Rural Development Edmonton, Alberta Agriculture, Edmonton, AB, Canada (retired),

³ HUN-REN-SZE PhotoPlant-Lab, Széchenyi István University, Mosonmagyaróvár, Hungary, ⁴ Department of Plant Sciences, Albert Kázmér Faculty of Mosonmagyaróvár, Széchenyi István University, Mosonmagyaróvár, Hungary, ⁵ Agricultural and Food Research Centre, Albert Kázmér Faculty of Mosonmagyaróvár, Széchenyi István University, Mosonmagyaróvár, Hungary

Lilies (*Lilium* spp.) are among the most important cut flowers, but fungal diseases can significantly reduce their yield and quality. In 2023, wilting symptoms were observed on lilies in a hobby garden in Hungary. Microscopic examination of symptomatic stem tissue revealed characteristic macroconidia of *Fusarium*. Based on conidial morphology and cultural characteristics, the single-conidial G300 isolate was identified as *Fusarium sporotrichioides*. Molecular identification based on the internal transcribed spacer region (ITS), translation elongation factor 1-alpha (*TEF1*), and the second largest subunit of the RNA polymerase II (*RPB2*) genes showed 99–100% sequence similarity to corresponding *F. sporotrichioides* sequences in GenBank. Phylogenetic analysis of the combined *TEF1* and *RPB2* sequences grouped G300 in one clade with other representative *F. sporotrichioides* isolates. Pathogenicity was confirmed by artificial inoculation of lily stems and detached leaves. Microscopic examination and sequencing verified the reisolated pathogen, fulfilling Koch's postulates. Under warm and moist conditions, *F. sporotrichioides* caused wilting and necrotic symptoms on stems and leaves, followed by downward disease progression, and symptomatic plants failed to recover.

Fusarium sporotrichioides has a broad geographical distribution and host range and is generally considered a weak plant pathogen. In contrast, the G300 isolate showed strong pathogenicity on lily stems and leaves, with disease severity comparable to that caused by *F. oxysporum* f. sp. *lilii*. As *F. sporotrichioides* has not previously been reported from the family Liliaceae, *Lilium* represents a new host for this species. Given that *F. sporotrichioides* is associated with cereals and that grain harvesting can substantially increase airborne spore concentrations, spores released from surrounding cereal fields during harvest may represent a potential infection risk for lilies. However, the role of this potential source of inoculum in lily infection remains to be investigated.

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Study on *Aspergillus flavus* bZIP-Type Transcription Factors focusing on fungicide sensitivity and aflatoxin production

Ágnes Kata Mondok^{1,2}, Barbara Brendzsák^{3,4}, Szilvia Kovács³, Tünde Pusztahelyi³, Tamás Emri^{1,5}, István Pócsi^{1,5,6} and Éva Leiter^{1,5}

¹ - Department of Molecular Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

² - Doctoral School of Pharmaceutical Sciences, University of Debrecen, Debrecen, Hungary

³ – Central Laboratory of Agricultural and Food Products, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen Debrecen, Hungary

⁴ - Doctoral School of Nutrition and Food Sciences, University of Debrecen, Debrecen, Hungary

⁵ - HUN-REN-UD Fungal Stress Biology Research Group, University of Debrecen, Debrecen, Hungary

⁶ - Food Chain Safety Laboratory Directorate, National Food Chain Safety Office, Budapest, Hungary

Aspergillus flavus is one of the most studied pathogenic filamentous fungi. Being a plant pathogen, it can cause significant damage in crop production, and is also widely known as human opportunistic pathogenic fungus causing the life-threatening disease aspergillosis. *A. flavus* also produces harmful secondary metabolites known as aflatoxins with carcinogenic and mutagenic properties.

The aim of our research was to study the importance and influence of global gene-expression regulator bZIP-type transcription factors on environmental stress-response, fungicide tolerance and aflatoxin production in *A. flavus*. We constructed five different bZIP deletion and complementation mutants, namely *Afap1*, *AflatfA*, *AflatfB*, *LziP* and *bZIP6*. In addition to the phenotypic characterization of the mutants we focused on the aflatoxin production ability of the strains cultured on maize kernels and solid agar media.

Our results showed that $\Delta Afap1$ and $\Delta AflatfA$ mutants were highly sensitive to oxidative stress generating agents, including H₂O₂, tBOOH and MSB, to the heavy metal stress triggering CdCl₂ and the cell wall integrity stress eliciting Congo Red. Their aflatoxin producing capability in infected corn kernels reduced significantly. The $\Delta LziP$ strain showed high sensitivity to the well known fungicide fludioxonil and CdCl₂, however exhibited stronger growth than the wild type strain in the presence of diamide. *bZIP6* deletion resulted in fungicide sensitivity to azoxystrobin and prothioconazole and remarkably reduced aflatoxin production in corn kernels.

According to our data *Afap1* and *AflatfA* may be promising targets for future antifungal therapies especially due to the rapidly changing environmental conditions. Further studies with *bZIP6* might also be considered in mycotoxin production on different grains and plants *in vivo*.

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Investigation of the virome of arable crops and associated weed species at a crop field

Gréta Mónos^{1,2}; Nikoletta Jaksa-Czotter¹, Éva Várallyay¹

¹ Genomics Research Group, Department of Plant Pathology, Institute of Plant Protection, Hungarian University of Agriculture and Life Sciences (MATE), Gödöllő, Hungary, ² Doctoral School of Natural Sciences, Doctoral Programme in Biological Sciences, Hungarian University of Agriculture and Life Sciences (MATE), Gödöllő, Hungary

Plant viruses can cause significant economic losses in agricultural crops. As potential virus hosts, weeds may contribute to the maintenance of plant viruses in agricultural ecosystems, thereby increasing the risk of infection in cultivated crops. The present study aimed to investigate plant viruses in an agricultural field located near Nagycsepely (Somogy County, Hungary) between 2024 and 2026, where maize, sunflower and wheat were grown in crop rotation.

Samples were collected at three sampling locations from both symptomatic and asymptomatic cultivated plants and weed species, including *Echinochloa crus-galli*, *Setaria viridis*, *Chenopodium hybridum*, *Chenopodium album*, *Datura stramonium*, *Convolvulus arvensis*, and *Sorghum halepense*. Following total nucleic acid extraction, the plant viromes were investigated using noncoding RNA high-throughput sequencing (HTS). The presence of viruses identified by HTS were confirmed by independent RT-PCR assays and Sanger sequencing.

During the 2024 sampling, a total of 63 samples collected from maize and seven weed species were examined, of which 22 tested positive for at least one plant virus. Wheat streak mosaic virus (WSMV), Barley virus G (BVG), Barley yellow dwarf virus (BYDV), and Wheat dwarf virus (WDV) were identified. WSMV was detected in maize and *E. crus-galli*, BYDV in maize and *S. viridis*, BVG in *E. crus-galli*, *S. viridis*, and *S. halepense*, while WDV was detected in *S. viridis* and *C. arvensis*.

In 2025, 52 weed samples collected from the sunflower field were analysed, with at least one virus detected in 27 samples. In addition to WSMV and BVG, Maize dwarf mosaic virus (MDMV) was also identified. WSMV and BVG were detected in *E. crus-galli* and *S. viridis*, while BVG was additionally confirmed in *C. album* and *Phragmites australis*. MDMV was detected in *E. crus-galli* and *S. halepense*.

During the 2026 sampling, 30 wheat samples were collected from three sampling locations, of which 29 tested positive for at least one virus. WSMV, BVG, BYDV, and WDV were detected in wheat samples. Investigation of the weed vegetation at the study site will continue following wheat harvest through sampling of the stubble vegetation.

Studying the same agricultural field over several years, we observed changes in its virome. Presence of economically important plant viruses in cultivated crops and weed species suggest that weeds may play an important role in the persistence of them. The study is ongoing, including the PCR-based confirmation of additional viruses identified by HTS and the investigation of weeds in the wheat stubble. The results may contribute to a better understanding of the relationships between plant viruses and their potential host plants in agricultural ecosystems, as well as the possible role of weeds as virus hosts and reservoirs.

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Arboviruses

Anna Nagy¹, Orsolya Nagy^{1,2}, Anita Koroknai¹, Nikolett Csonka¹, Anna Rebeka Kálmán¹, Katalin Szomor³, Erzsébet Barcsay³, Mária Takács^{2,3}

1 National Reference Laboratory for Viral Zoonoses, National Center for Public Health and Pharmacy, Budapest, Hungary

2 Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary

3 Division of Microbiological Reference Laboratories, National Center for Public Health and Pharmacy, Budapest, Hungary

Arboviruses – arthropod-borne viruses – comprise a diverse group of viruses transmitted primarily by mosquitoes and ticks and include major human pathogens responsible for febrile, neurological, haemorrhagic and other clinical manifestations. Their global public health impact is substantial, driven by increasing geographic distribution of vectors, climate and environmental changes, international travel and trade, and the emergence or re-emergence of viral diseases. Accurate laboratory diagnosis is therefore essential not only for patient management, but also for surveillance, outbreak detection and public health response.

The presentation provides an overview of selected arbovirus infections of relevance to Hungary, with particular emphasis on endemic infections and viruses that may be imported through international travel. In Hungary, the microbiological differential diagnosis of these infections is performed at the National Reference Laboratory for Viral Zoonoses of the National Center for Public Health and Pharmacy, where a broad range of serological and molecular diagnostic methods is available.

The epidemiological overview will be presented by diagnostic data from the National Reference Laboratory. Between 2016 and 2025, 233 dengue, 19 chikungunya and 13 Zika virus infections were diagnosed, while no Oropouche, Mayaro or yellow fever cases were identified. One recent Japanese encephalitis virus infection was also detected. All imported infections were associated with a documented history of travel to endemic areas. For 2016–2025, 502 locally acquired and 18 imported West Nile virus infections were diagnosed, together with 212 tick-borne encephalitis virus infections. Since the introduction of diagnostic testing in 2018, four autochthonous Usutu virus infections have been identified.

The main focus of the presentation will be the laboratory differential diagnosis of arbovirus infections, including the appropriate use and interpretation of molecular and serological assays. Particular attention will be paid to diagnostic challenges such as the short period of viraemia, the timing and type of clinical specimens, variable antibody kinetics, cross-reactivity between related orthoflaviviruses, and the interpretation of positive or borderline serological results.

Applied industrial big data analytics to optimise fermentation in TAPI

István Nagy¹, Gergő Kertész¹, Zoltán Németh²

¹MaxFlow Kft, Budapest, Hungary-² TAPI Hungary Industries Ltd., Debrecen, Hungary

TAPI is a leading API producer with a significant fermentation production capacity. The fermentation processes are inherently variable, exhibit long time delays, and involve complex, multi-variable dependencies. Maintaining a precise substrate level is critical throughout the extended fermentation periods, often exceeding 200 hours, despite this complex environment. To address these challenges, a co-operation project with an external partner was initiated to develop a novel control strategy for substrate dosage. This strategy incorporates big data analytics, a soft-sensor for real-time substrate concentration prediction and continuous concentration control.

TAPI introduced automatic substrate feeding technology into the process control system, which calculates the optimal feeding rate using real-time data from the process. The creation of the automatic feeding system required a unique application and new solutions to compensate and manage the disturbances that are typical of bioprocesses. By using the automatic feeding rate, TAPI was able to achieve a significant productivity increase by keeping the substrate concentration at an optimal and very precise level during the process. Additionally, the fluctuation of reproducibility was minimized, which is a typical behavior of the bioprocess production. Further advantages of the improvement are the decrease of carbon footprint, environmental emissions, and waste disposal.

HTLV the first human retrovirus:receptor preference (In memoriam Robin Weiss 1940-2026)

Károly Nagy

Semmelweis University, Budapest, Hungary

The human T lymphotropic virus (HTLV) has been isolated at the end 20th century. The virus is endemic in Japan, the Caribbean region including south east USA and in some part of Africa inducing severe T-cell leukemia. In the characterization of the new virus Robin Weiss had an outstanding role. He was then the director of Institute of Cancer Research in London, and head of the Chester Beatty Laboratories. As a scientist working years in his team, our research focused on the biological characterization of HTLV. First we demonstrated, that sera of infected patients from USA, Japan and Africa equally neutralize the virus isolated from various geographical regions, meaning that it has a single serotype. Then we studied immigrants from the Caribbean regions into London confirming the presence of HTLV in Europe. Employing an XC assay, we showed that HTLV is able to induce syncytia (multinucleated giant cells) in several monolayer cell lines in vitro, and the sera of infected patients are able to inhibit it (syncytia inhibition assay), providing a simple in vitro detection assay. We developed an in vitro retrovirus pseudotype assay (using HTLV/VSV pseudovirions) to study HTLV cellular receptor preference. Based upon these results our most important discovery was to demonstrate that HTLV can infect not only suspension T-lymphocytes, but several monolayer cells as well in vitro. These pioneering retrovirus research – published in leading scientific papers, including *Science* – helped shaped both virology and cancer research. Robin Weiss later joined the University College of London as Professor of Viral Oncology and Director of the Wohl Virion Centre until his retirement. Professor Robin Weiss is recognized as one of the most influential scientists in modern virology and cancer biology, passed away on 27 February 2026.

Efforts towards the establishment of a unified EU-level environmental AMR surveillance system (EARS-Env)

Ábel Csongor Németh^{1,2}, Eszter Róka¹, Bernadett Khayer¹, Balázs Seres^{1,2}, Boglárka Pollák¹, Eszter Schuler¹, Krisztina Kurucz-Lajtos¹, Márta Vargha¹

¹National Center for Public Health and Pharmacy, Department of Public Health Laboratories and Methodology, Budapest, Hungary ²Doctoral School of Environmental Sciences, Eötvös Loránd University, Budapest, Hungary

The second phase of EU-JAMRAI 2 has set the ambitious goal of assessing the problem of antimicrobial resistance (AMR) and developing risk mitigation strategies in cooperation with 30 EU Member States and 120 partner institutions. Operating across multiple sectors (including human health, animal health, and environmental health) the project aims to support the fight against antimicrobial resistance within the framework of the "One Health" approach.

One of the primary objectives within the environmental sector is the establishment of a pilot EARS-Env (European Antimicrobial Resistance Surveillance network for the Environment), tasked with providing complementary data alongside the already operational EARS-Net and EARS-Vet systems. The process began with defining the objectives of the pilot surveillance system, laying a solid foundation for appropriate data collection regarding resistance circulating in the covered population, the impacts of wastewater treatment, and potential risks to bathers through the examination of selected sampling sites and targets. The pilot EARS-Env system relies on the combined application of culture-based and quantitative molecular (qPCR) techniques, which will be supplemented by metagenomic and whole-genome sequencing, thereby enabling the adaptive operation of the surveillance panel. The results of the molecular analyses are expected after November 2026, complementing the summer data from the culture-based analyses. According to the culture-based results, a significant difference in AMR load (both for ESBL-producing *E. coli* and carbapenem-resistant *Enterobacterales*) is observed between the two monitored wastewater treatment plants that utilize nearly identical treatment technology (tertiary treatment stage for nitrogen and phosphorus removal) but serve vastly different population numbers (approx. 100k p.e. and 1.300k p.e.). For the monitored bathing waters, the ESBL load originating from coliform bacteria was negligible at both locations. In the future, this tripartite methodology is expected to provide reliable and goal-oriented data for the professional community and decision-makers alike, supporting the practical implementation of the revised Urban Wastewater Treatment Directive (EU 2024/3019). This presentation focuses on highlighting the Hungarian aspects of the project, which was implemented with the support of EU-JAMRAI 2 (No. 101127787).

IBSx: Extending Identity-by-State analysis for *Saccharomyces* phylogenomics and validation using *in silico*-generated test genomes

Balint Nemeth^{1,2}, Valter P. Pfliegler²

¹ Department of Molecular Biotechnology and Microbiology, University of Debrecen, Debrecen, Hungary ² Doctoral School of Nutrition and Food Sciences, University of Debrecen, Debrecen, Hungary

Phylogenomic studies of *Saccharomyces* employ a range of approaches to infer evolutionary relationships among strains. At the population level, commonly used workflows rely on reference-based read mapping, followed by genetic distance estimation and the construction of Neighbor-Joining (NJ) or maximum-likelihood (ML) trees. Frequently used tools include the SNPRelate R package, which converts VCF files into GDS format for NJ tree construction, and IQ-TREE, which generates ML trees from SNP matrices. The resulting phylogenetic relationships are typically visualized using the ape R package or SplitsTree. However, the genomic complexity of *Saccharomyces* poses substantial challenges for these approaches, particularly in population-level analyses. Highly polyploid and heterozygous genomes, together with missing genomic regions, such as those frequently observed in interspecies hybrids, can result in inaccurate sample placement and distorted branch lengths. Moreover, most existing approaches focus exclusively on biallelic SNPs, thereby excluding potentially informative multiallelic and other variant sites.

Here, we present IBSx, an extension of the standard Identity-by-State (IBS) framework. IBSx performs pairwise comparisons of genotype data directly from multisample VCF files and quantifies shared alleles according to the standard IBS classification. Alleles that cannot be compared due to missing data are assigned to a novel category, IBSx, allowing the extent of missing data to be quantified and analyzed independently. Pairwise genomic distances are calculated as:

$$\text{distance} = 1 - (\text{IBS1} \times 0.5 + \text{IBS2} \times 1) / (\text{IBS0} + \text{IBS1} + \text{IBS2})$$

The resulting distance matrix can be used for both Neighbor-Net network construction and Neighbor-Joining tree estimation. As IBSx operates directly on the GT field of the VCF, it naturally accommodates multiallelic sites and enables the joint analysis of SNPs and INDELs.

We evaluate the performance and robustness of IBSx using *in silico*-generated short-read genomes simulated with NGSNGS. The benchmark dataset was designed to mimic natural *Saccharomyces* populations and incorporates variation in ploidy, heterozygosity, interspecies hybridization, and SNP and INDEL profiles. We compare IBSx with SNPRelate and IQ-TREE and additionally include an Assembly- and Alignment-Free approach as an independent benchmark. Our results demonstrate that IBSx provides a more accurate and sensitive framework for phylogenomic analyses of genetically complex organisms such as *Saccharomyces*. In addition, the simulated benchmark population generated in this study provides a useful resource for the development and validation of future phylogenomic methods.

The tomato powdery mildew fungus and its close relatives - One species or many?

Márk Z. Németh¹, Uwe Braun², Michael Bradshaw^{3,4}

¹ HUN-REN Centre for Agricultural Research, Plant Protection Institute, Budapest, Hungary, ² Martin Luther University, Institute of Biology, Department of Geobotany and Botanical Garden, Herbarium, Halle (Saale), Germany, ³ North Carolina State University, Center for Integrated Fungal Research, Department of Entomology and Plant Pathology, Raleigh, NC, USA, ⁴ Harvard University, Farlow Herbarium, Cambridge, MA, USA

Powdery mildew is one of the most common diseases of angiosperms and is caused by obligate biotrophic pathogenic fungi in the family Erysiphaceae. Host range varies considerably among species. Some species are restricted to host plants of a single plant genus (monophagous), whereas others can infect hosts belonging to multiple plant genera or even multiple plant families (oligo- and polyphagous, respectively). Host range has also been an important criterion for delimiting powdery mildew species.

The tomato-infecting powdery mildew fungus *Erysiphe neolycopersici* and its close relatives, including *E. sedi*, *E. takamatsui*, *E. caricae-papayae* and others, were originally described as separate species, in part based on their host plants.

However, analyses of the internal transcribed spacer (ITS) region, which is widely used in fungal taxonomy, revealed that taxa considered distinct based on their host associations may have identical or highly similar ITS sequences. Consequently, the term *E. aquilegiae* complex was introduced, which also included the tomato powdery mildew fungus. Using a broad species concept, the taxa within the complex were traditionally considered to comprise a single, highly polyphagous species, *E. aquilegiae*.

Recent multilocus phylogenetic analyses have provided improved resolution of species boundaries and their relationship to host specificity. The most comprehensive reassessment of the *E. aquilegiae* complex, based on eight loci, recognizes two widespread, highly polyphagous species with partly overlapping host ranges, *E. aquilegiae* s. str. and *E. hortensiae*, as well as several more specialized species.

These results demonstrate that host range can vary considerably among taxa within a powdery mildew species complex and that host range alone is insufficient to delimit species boundaries. Reliable species delimitation therefore requires integrating molecular, host range and other biological data.

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Sodium acetate modulates colony development and amylase activity in *Aureobasidium pullulans* strain Y37: possible evidence of a cross-species acetate-dependent quorum sensing-like response

Ádám Novak¹, Dóra Szabó¹, Nikolett Molnár¹, Kálmán Zoltán Váczy¹, Zoltán Karácsony¹

¹ Eszterházy Károly Catholic University, Food and Wine Research Institute, Eger, H3300, Hungary

² Hungarian University of Agriculture and Life Sciences, 2100 Gödöllő, Páter Károly street 1

Aureobasidium pullulans is a cosmopolitan, black, dimorphic fungus with ecological and biotechnological importance. In our previous work, we demonstrated its positive role in the development of grapevine esca disease through an interaction with the pathogenic fungus *Phaeomoniella chlamydospora*. Quorum sensing (QS) in fungi allows cell density-dependent coordination of morphogenesis, biofilm formation, and metabolism. Our previous studies identified acetate as a potential QS molecule regulating development and biofilm formation in *P. chlamydospora*. We hypothesized that acetate may also trigger similar responses in the case of *A. pullulans*. The aim of the present study is to investigate the acetate dependence of the interaction between the two fungal species as an interspecific QS-like response.

The response of *A. pullulans* strain Y37 to a two-fold dilution series of nine concentrations of sodium acetate (0.78–100 mM) was examined on potato dextrose agar with three colonies per plate for nine days at 25 °C. To separate the acetate-specific effects from the effects of sodium, NaCl treatments providing the same sodium concentration were used as controls. The area of the colonies and the degree of sporulation were measured. Extracellular amylase activity was visualized with Gram's iodine solution, and the resulting hydrolysis zones were measured directly or normalized to the colony area. The data were analyzed by ordinary one-way ANOVA followed by Tukey's multiple comparison test. Sodium acetate significantly affected colony growth and all amylolytic parameters. Increasing acetate concentration inhibited radial growth while inducing sporogenesis. In parallel, the size of the starch hydrolysis zone and its ratio to the colony surface area increased. The corresponding NaCl controls resulted in weaker or different responses, suggesting that the observed changes are not solely attributable to the effect of sodium. The observed effects of acetate are more indicative of a coordinated reorganization of colony development and enzyme expression, rather than a simple stimulatory or inhibitory effect.

Our results support the hypothesis that acetate may act as a regulatory metabolite in *A. pullulans* Y37 strain, thus mediating the effects of *P. chlamydospora* on *A. pullulans*. The nonlinear, concentration-dependent response is consistent with an acetate-mediated, QS-like mechanism. To confirm whether acetate truly functions as a QS molecule, further studies need to be carried out to examine endogenous acetate production and cell density-dependent gene expression.

The research was supported by the TKP-NKTA-16 research project.

First Results of RSV Amplicon-Based Whole-Genome Characterization from Hungarian Samples in 2026

Bereniké Novák¹, Zsolt Czira², Júlia Sarkadi², Éva Hercegh², Katalin Tóth-Ihász ², Erzsébet Barcsay², Zoltán Kis¹, Bernadett Pályi¹, Judit Henczkó ¹

1 Department of National Biosafety Laboratory, National Center for Public Health and Pharmacy, H-1097 Budapest, Hungary

2 Department of Virology, National Center for Public Health and Pharmacy, H-1097 Budapest, Hungary

Human orthopneumovirus (Respiratory syncytial virus, RSV) is a major cause of acute lower respiratory tract infections, particularly in children under five years of age and older adults, and represents a substantial seasonal burden on healthcare systems. RSV is classified into two major antigenic subgroups, RSV-A and RSV-B, which can be further divided into lineages based on their genetic diversity. Genomic characterization of circulating RSV strains is an important component of molecular surveillance, providing information on viral diversity and evolution. Previously, RSV genotyping in our laboratory was performed using metagenomic sequencing. To establish a more targeted approach for RSV-positive samples, an amplicon-based whole-genome sequencing workflow was implemented with the aim of replacing metagenomic sequencing for routine genomic characterization of RSV. A total of 44 RSV-positive respiratory samples collected between January and April 2026 were selected from specimens submitted in Hungary through different respiratory surveillance programmes (severe acute respiratory infections influenza-like illness), outbreak investigations, and routine diagnostic testing. Samples with RT-qPCR Ct values below 26 were included, comprising 20 RSV-A and 24 RSV-B samples. Following reverse transcription, cDNA was amplified using RSV-A- and RSV-B-specific ARTIC-like primer sets consisting of two pools of overlapping amplicons designed to cover the RSV genome. The amplicon-based workflow was incorporated into the Illumina COVIDSeq Test library preparation protocol. Sequencing was performed on the Illumina MiSeq platform using MiSeq Reagent Kit v2 chemistry. Bioinformatic analysis was performed using a command-line workflow with CUDA-CAP and MIRA.

Of the 44 RSV-positive patients, 72.7% were children under five years of age, and 25% were hospitalized in non-critical care units. Based on the initial RT-qPCR results, 20 samples were classified as RSV-A and 24 as RSV-B. Successful lineage assignment by whole-genome sequencing was achieved all initially identified RSV-A and RSV-B samples. Eight different RSV-A lineages were identified and among the successfully assigned RSV-B samples, three lineages were identified. The identified RSV-A and RSV-B lineages were consistent with those circulating in Europe during the study period. One RSV-B-positive patient had a viral coinfection with SARS-CoV-2. RSVsurver analysis detected no F-gene mutations associated with reduced susceptibility or resistance to nirsevimab, palivizumab or clesrovimab. These results demonstrate that amplicon-based WGS represents a reliable targeted alternative to metagenomic sequencing for the routine genomic characterization of RSV-positive surveillance samples. The targeted amplification approach may enable successful characterization of samples with lower viral loads and higher Ct values, thereby increasing the number of samples suitable for genomic surveillance compared with metagenomic sequencing. Its implementation is in line with European efforts to strengthen RSV genomic surveillance and may contribute to the further development of integrated genomic surveillance. This work was supported by the HERA2 project, co-funded by the European Union under the EU4Health Programme (grant agreement No. 101102440).

Fungal community dynamics across management treatments over time and environmental drivers in Hungarian oak–hornbeam forests

Kennedy Ododa^{1,2}, Péter Ódor^{3,4}, Bence Kovács³, Flóra Tinya³, Réka Aszalós³, Carla Mota Leal^{1,2}, Adrienn Geiger^{1,2}, Anna Molnár^{1,2}, András Bidló⁴, József Geml¹

¹Environmental Microbiome Research Group, Eszterházy Károly Catholic University, Leányka u. 8, 3300 Eger, Hungary

²Doctoral School of Natural Sciences, Hungarian University of Agricultural Sciences, Páter Károly u. 1, 2100 Gödöllő, Hungary

³HUN-REN Centre for Ecological Research, Institute of Ecology and Botany, Alkotmány út 2-4, 2163 Vácrtót, Hungary

⁴University of Sopron, Faculty of Forestry, Institute of Environmental Protection and Nature Conservation, Bajcsy-Zsilinszky u. 4, Sopron 9400, Hungary

Fungi are key players in nutrient cycling, plant-fungal interactions and forest health, but the effects of different silvicultural intensities on soil fungal community changes remain insufficiently resolved. We investigated the taxonomic and functional responses of soil fungi to contrasting forest management treatments in the Pilis Forestry Systems Experiment, a temperate oak-hornbeam forest in Hungary. Samples were taken from 30 experimental plots in autumn 2020 and 2021, representing five silvicultural treatments (clear-cutting, gap-cutting, preparation cutting, retention tree groups, and an untreated control) six and seven years after treatment, respectively. We employed high-throughput ITS2 DNA metabarcoding to characterize soil fungal communities and their responses were assessed across major functional guilds, including ectomycorrhizal fungi (ECM), saprotrophs, plant pathogens, wood decomposers, mycoparasites, root endophytes and animal parasites. The fungal community composition was consistently driven by silvicultural treatments, with significant effects detected for all functional guilds ($R^2 = 8.97\text{--}15.37\%$, $p < 0.001$). However, fungal guilds differed significantly in their response to management intensity. ECM richness decreased most strongly in the clear-cutting, while preparation cutting and retention treatments showed relatively moderate responses. By contrast, saprotrophic fungi showed increased richness with clear-cutting in the year 2020 but decreased richness in 2021, and plant pathogens, wood decomposers and mycoparasites showed increased richness with more intensive disturbance. Root endophytes and animal parasites were relatively stable across silvicultural practices. Community composition was also related to microclimatic conditions, especially soil temperature and moisture variables ($R^2 = 6.23\text{--}12.67\%$, $p < 0.01$). Significant yearly changes showed that fungal communities did not respond consistently through time, with the year 2021 being notably drier compared to 2020, hence associated with alternative successional trajectories among functional guilds. Indicator species analysis showed management-associated taxa, such as ECM genera *Inocybe* and *Russula* under less disturbed conditions, while saprotrophic and pathogenic taxa were more strongly associated with severely managed treatments e.g., clear cuts. Our results show that the management intensity does not respond to fungi consistently, but rather alters a forest fungal community in guilds and taxa specific pattern. These findings underline the importance of forest structure and the abiotic environment in shaping the community dynamics of fungal communities, which may in turn affect forest health and productivity.

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Enhancement of horsemint essential oil antifungal activity against the phytopathogenic fungus *Botrytis cinerea* by the use of a pine terpene-based adjuvant

Xénia Karácsony-Pálfi¹, Nóra Bakos-Barczi¹, Marietta Korózs¹, Kálmán Zoltán Váczy¹, Zoltán Karácsony¹, Katalin Patonay¹

¹Eszterházy Károly Catholic University, Food and Wine Research Institute, Leányka Str. 8., Eger, H3300, Hungary

Viticulture is one of the most spray-demanding agricultural sectors, mostly because of the management of several fungal diseases affecting the grapevine. In this context, one of the most relevant syndromes is grey rot, caused by the ascomycetous fungus *Botrytis cinerea*. The extensive use of synthetic fungicides in recent decades has led to environmental and medical concerns, as well as the development of resistant fungal strains. The use of natural antifungals would solve these problems. Plant essential oils are ideal candidates for this purpose because of their high antifungal efficiency against a wide spectrum of fungi. However, their agronomical application is limited by their high volatility and hydrophobicity, which problems can be overcome through the use of appropriate adjuvants. In the present study, the combined effects of the essential oil of horsemint (*Mentha longifolia*) Hór-1 chemotype and a pine terpene-based adjuvant were investigated against *B. cinerea*.

The antifungal activity of the essential oil was investigated in the 0.1-0.055% (v/v) interval by examining the colony-forming ability of *B. cinerea* conidia on intoxicated solid media. A fungicidal effect was observed only in the 0.085-0.1% (v/v) concentration range, while in the presence of 0.1% (v/v) pine terpene adjuvant, similar fungitoxicity was observed at 0.055% (v/v). Microscopic examination of treated fungal spores after methylene blue staining reinforced the devastating effect of the essential oil-adjuvant combination; >90% of the spores were dead. Fluorescently labelled essential oil showed cytoplasmic and/or plasmamembrane localization in *B. cinerea* hyphae, and this affinity was possibly promoted by the adjuvant.

According to our results, the antifungal activity of horsemint essential oil against *B. cinerea* can be greatly enhanced by a pine terpene adjuvant, and this formulation is possibly applicable in the field.

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The newest Public Health Emergency of International Concern: The Bundibugyo outbreak in Democratic Republic of Congo and the role of the European Public Health Reference Laboratory network

Bernadett Pályi^{1,2,3}, Dániel Déri^{1,2,3}, Bereniké Novák^{1,2}, Gyula Zsdei^{1,2}, Zoltán Kis^{1,2,3}

¹ National Biosafety Laboratory, National Center for Public Health and Pharmacy, Budapest, Hungary, ² European Union Public Health Reference Laboratories for Emerging, Rodent-borne and Zoonotic Viral Pathogens (EURL-PH-ERZV), ³ European Research Infrastructure on Highly Pathogenic Agents (ERINHA-AISBL), Brussels, Belgium.

Bundibugyo virus (*Bundibugyo ebolavirus*, BDBV)—a high-consequence filovirus—causes severe Ebola virus disease characterized by high fatality rates and potential for rapid escalation. Until recently, only two outbreaks were recorded in the history of BDBV. On 17 May 2026, the outbreak of Ebola disease caused by Bundibugyo virus (BDBV) in the Democratic Republic of the Congo (DRC) and Uganda was declared a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO) and a Public Health Emergency of Continental Security (PHECS) by Africa CDC. Due to delays in proper diagnostic methodology, the outbreak was recognised only after a prolonged period of transmission

The European Union Reference Laboratory for Public Health on Emerging, Rodent-borne and Zoonotic Viral Pathogens (EURL-PH-ERZV), coordinated by Sweden, Hungary, Italy and France, was established by the European Commission to strengthen laboratory preparedness for high-consequence viral pathogens across the European Union. It supports national reference laboratories through the harmonisation of diagnostic methods, provision of reference materials, external quality assessments, training, and technical expertise.

Recent preparedness and diagnostic capacity building and vaccine research have predominantly been focused on Ebola virus (EBOV, previously Zaire ebolavirus). The recent outbreak, with the delayed recognition of the Bundibugyo virus disease outbreak in the DRC, underscored gaps in preparedness for less frequently encountered orthoebolaviruses. The international response to the active Bundibugyo virus disease (BVD) outbreak is driven by a coordinated multi-agency effort across research, financing, logistics, and field operations. With the coordination and guidance of WHO, diagnostics, logistics, and clinical therapeutics are helping the affected health zones. Coalition for Epidemic Preparedness Innovations and Gavi, the Vaccine Alliance, by activating funds and the 100 Days Mission program, are already testing vaccines against the BDBV. The EURL-PH-ERZV is actively involved in EU preparedness by assessing global and European laboratory readiness for BDBV, evaluating and comparing commercial and reference RT-qPCR assay performance against published BDBV strains. Together with the WHO Regional Office for Europe conducted a rapid mapping survey to assess diagnostic preparedness for BDBV across Europe, to identify current testing capacity, potential gaps, and priorities for coordinated preparedness activities and targeted support.

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Characterization of a negatively charged defensin from *Solanum lycopersicum* L.

Rebeka Papp^{1,2}, Zsüliett Pfeffer¹, Liliána Tóth¹, Attila Borics³, Zoltán Kele⁴, Györgyi Váradi⁴
László Galgóczy¹

¹ Department of Biotechnology and Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary,

² Doctoral School of Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

³ Institute of Biochemistry, HUN-REN Biological Research Centre, Szeged, Hungary

⁴ Department of Medical Chemistry, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary

Plant defensins are cysteine-rich antimicrobial peptides that play an important role in plant defense and have attracted considerable interest as potential alternatives for controlling fungal infections in agriculture. They are typically amphipathic and exhibit broad-spectrum antimicrobial activity against bacteria, yeasts, and filamentous fungi. Most characterized plant defensins are positively charged, whereas negatively charged defensins remain comparatively poorly characterized, with limited information available on their structural properties and biological functions. In the present study, we investigated the structural characteristics and antimicrobial activity of the negatively charged K4CV21 from *Solanum lycopersicum* L. The structural properties of K4CV21 were examined by *in silico* modelling. The recombinant protein was produced in a *Komagataella phaffii* heterologous expression system. Electronic circular dichroism (ECD) spectroscopy and mass spectrometry (MS) were used to characterize the recombinant protein and confirm the successful production of mature K4CV21. The antimicrobial activity of recombinant K4CV21 was assessed using susceptibility and spot assays against plant- and human-pathogenic bacteria (*Erwinia mallotivora*, *Pseudomonas syringae*, and *Xanthomonas campestris*), yeasts (*Candida* spp.), and filamentous fungi (*Aspergillus* spp., *Botrytis cinerea*, and *Fusarium oxysporum*). The *in silico* modelling revealed that K4CV21 is an amphipathic molecule with a negative net charge and a secondary structure characteristic of plant defensins, consisting of a cysteine-stabilized $\alpha\beta$ motif. ECD spectroscopy supported the presence of disulfide bonds as well as α -helical and β -sheet structural elements. MS confirmed the successful production of mature K4CV21 in the heterologous expression system, although an additional EAEA repeat remained at the N-terminus of the recombinant protein. However, neither the susceptibility nor the spot assays revealed detectable antimicrobial activity against any of the tested microorganisms. These findings indicate that, despite sharing structural characteristics with antimicrobial plant defensins, K4CV21 does not exhibit detectable direct antimicrobial activity under the investigated conditions. The absence of detectable antimicrobial activity suggests that K4CV21 may contribute to plant defense through mechanisms distinct from the direct antimicrobial activity, typically associated with positively charged defensins. Further studies, including transcriptional analyses, are required to elucidate potential indirect or immunomodulatory functions of K4CV21 in plant defense.

Comprehensive Metagenomic Analysis of Phytoplasma-Infected Apple and Apricot Orchards

Lilla Dorottya Péri^{1,2}, Zsuzsanna Nagyné Galbács¹, Éva Várallyay¹

¹ Genomics Research Group, Department of Plant Pathology, Institute of Plant Protection, MATE, Gödöllő, Hungary ² Doctoral School of Natural Sciences, Doctoral Program of Biological Sciences, MATE, Gödöllő, Hungary

Over the last decade, apple (*Malus domestica*) and apricot (*Prunus armeniaca*) orchards in Hungary, have shown alarming decline. While phytoplasmas are recognized as major causative agents of the mortality (in some cases exceeding 50% in apricot), symptom complexity suggests that additional biotic and abiotic factors could be involved. In this exploratory study, we compared microbiome of symptomatic and asymptomatic trees from an apricot (Somogytúr) and an apple (Olcsavapáti) orchard in Hungary. The study combined colourimetric LAMP, nested PCR, small RNA (sRNA) sequencing and LoopSeq long-read sequencing, to investigate the presence of phytoplasmas, determine the viromes and characterize microbial communities of the trees. Phytoplasma DNA was detected in both symptomatic and asymptomatic plants, highlighting latent infections. Analysis of sRNA profiles revealed distinct viromes of the trees. Apple trees were infected by Citrus concave gum-associated virus, apple rubbery wood virus 2, and Apple hammerhead viroid, while apricot trees mainly harboured cherry virus A and hop stunt viroid. The presence of viruses and viroids were neither uniform nor could it be linked to the symptoms. Microbiome analysis revealed diverse fungal and bacterial communities in both hosts. Given the exploratory nature of this research and limited sample size, changes in the microbial composition did not reach statistical significance; nonetheless, biological trends emerged across both hosts. In apple, 72 bacterial and 43 fungal genera, while in apricot, 49 bacterial and 14 fungal genera were identified, respectively. In apple, among bacteria, *Candidatus Phytoplasma mali* was detected exclusively in symptomatic samples. Fungal genera in the symptomatic trees were dominated by the entomopathogenic fungus *Cordyceps* genus, where *Podosphaera*, the causal agent of powdery mildew, and its hyperparasite *Ampelomyces* were also present. Phyllosphere-associated yeasts *Vishniacozyma* and *Derxomyces* were more prevalent in asymptomatic trees. *Candidatus Phytoplasma prunorum* was detected in the symptomatic apricot samples, together with *Cordyceps species*. and *Microbacteriaceae* and *Erwiniaceae* taxa, whereas *Carnobacteriaceae* was more abundant in asymptomatic trees. Among the fungal taxa, *Holtermanniales* and *Cantharellaceae* were associated with symptomatic trees, while *Russulaceae* was more abundant in asymptomatic trees. Collectively, this pilot study indicates that although orchard decline can be connected to the phytoplasma infection, the syndrome can be shaped by the combined effects of phytoplasmas, viruses and, viroids and altered bacterial and fungal communities, which can be further affected by the genotype of the host and environmental stresses

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Nucleocytoplasmic localization of CMV and PSV 2b Proteins Contributes to RNA Silencing Suppression and Viral Pathogenicity

Dóra Pinczés¹, Réka Sáray¹, Attila Fábián², Asztéria Almási¹, Katalin Salánki¹

¹ Department of Plant Pathology, Plant Protection Institute, Centre for Agricultural Research HUN-REN Budapest, Hungary

² Department of Biological Resources, Plant Protection Institute, Centre for Agricultural Research HUN-REN Martonvásár, Hungary

The intracellular distribution of viral proteins between the nucleus and cytoplasm can be a critical determinant of their biological activity. The role of both nuclear and cytoplasmic localization of cucumovirus 2b proteins, have been described in distinct aspects of RNA silencing suppression (RSS) and pathogenicity, suggesting that an appropriate nucleocytoplasmic balance rather than exclusive localization to either compartment may be important for optimal function. Here, we investigated this relationship using the 2b proteins of cucumber mosaic virus (CMV) and peanut stunt virus (PSV), together with recombinant proteins in which N-terminal and/or nuclear localization signal (NLS)-containing regions were exchanged between the two viruses. CMV 2b displayed a relatively balanced nuclear–cytoplasmic distribution and strong RSS activity, whereas PSV 2b was localized predominantly in the nucleus and exhibited weaker suppressor activity. Interestingly, replacement of the NLS regions or N-terminal region alone did not substantially alter the localization pattern. In contrast, combining the exchanged NLS-containing and the preceding N-terminal regions resulted in pronounced changes in subcellular localization. The NLS P/C recombinant exhibited a more balanced distribution and the highest intracellular homogeneity among the tested proteins, accompanied by strong RNA silencing suppressor activity and enhanced disease symptoms on *Nicotiana benthamiana* when introduced into CMV background. Conversely, the predominantly nuclear NLS C/P protein showed markedly reduced suppressor activity, and the infectious clone caused mild symptoms on *N. benthamiana*. AlphaFold modelling further revealed conserved predicted structural regions among the four proteins, including the first predicted three-dimensional model of PSV 2b. Together, these findings suggest that the biological activity of 2b is influenced not simply by nuclear targeting, but by the precise nucleocytoplasmic distribution established through the combined action of multiple trafficking determinants.

Two novel lineages and new records of root-colonizing dark septate endophytes in natural and agricultural habitats

Alexandra Pintye^{1,2}, Ildikó Imrefi², Fruzsina Matolcsi^{1,2}, Orsolya Molnár¹, Tamás Árendás³, Petra Lengyel², Ruth Rogers², Péter Sasvári⁴, Márta Krasznai^{5,6}, Janka Emília Cserős^{1,2}, Sándor Csikos², Miklós Lovas^{7,8}, Imre Boldizsár^{2,6,9}, Gábor M. Kovács^{1,2,10}

¹ Plant Pathology Department, Plant Protection Institute, Centre for Agricultural Research, HUN-REN, Budapest, Hungary, ² Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Budapest, Hungary, ³ Agricultural Institute, Centre for Agricultural Research, HUN-REN, Martonvásár, Hungary, ⁴ Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden, ⁵ Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary, ⁶ Center for Pharmacology and Drug Research & Development, Semmelweis University, Budapest, Hungary, ⁷ Centre for Research and Development, Eszterházy Károly Catholic University, Eger, Hungary, ⁸ Hevesy György PhD School of Chemistry, Institute of Chemistry, Eötvös Loránd University, Budapest, Hungary, ⁹ Department of Pharmacognosy, Semmelweis University, Budapest, Hungary ¹⁰ HUN-REN-ELTE-MTM Integrative Ecology Research Group, Budapest, Hungary

Root-colonising endophytic fungi, including dark septate endophytes (DSEs), are common members of plant-associated microbial communities and play important ecological roles, especially under environmental stress. In this study, we examined the diversity and characteristics of root-associated endophytic fungi occurring in natural grasslands and agricultural croplands in Hungary. The roots of *Festuca vaginata* and *Stipa borysthénica* were sampled in semiarid grasslands near Fülöpháza, while *Triticum aestivum* and *Zea mays* roots were collected from wheat and corn croplands at the Centre for Agricultural Research in Martonvásár. Isolates were obtained from surface-sterilized healthy roots, and representative isolates were characterized morphologically. Resynthesis experiments were performed, and genomic DNA was extracted, followed by multilocus phylogenetic analyses.

The phylogenetic analyses showed representatives of several fungal families, including *Phaeosphaeriaceae*, *Magnaporthaceae*, *Chaetomiaceae*, and *Periconiaceae*. Two previously unrecognized fungal lineages were identified and are formally introduced as *Ophiosphaerella multiglobosa* sp. nov. and *Xeropratinus graminum* gen. et sp. nov. The latter representing a newly established monotypic genus within *Chaetomiaceae*. We also recovered isolates corresponding to recently described species, including *Polydomus karssenii*, *Magnaporthiopsis stipae*, *M. cynodontis* and *Periconia neomacrospinosa*. Their occurrence in both natural and agricultural ecosystems provides new records that expand current knowledge of their distribution and host associations.

In vitro resynthesis experiments confirmed the endophytic nature of all examined taxa. The fungi colonized internal and external root regions without detectable negative effects. Metabolite profiling revealed distinct chemical patterns among the investigated lineages, and several known bioactive compounds. In addition, as well as a potentially previously unreported metabolite in *Xeropratinus graminum*. Overall, our results indicate that newly discovered and recently characterized DSE taxa have a broader ecological distribution and greater environmental versatility than previously assumed. The findings highlight the value of combining phylogenetic, ecological, experimental, and metabolomic approaches to better understand the diversity, biology, and functional significance of root-associated fungal communities.

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Agrobacterium tumefaciens-mediated transformation (ATMT) of filamentous fungi

István Pócsi^{1,2}, László Kozák¹, Anita Király¹, Sandugash Ibragimova^{1,2}, Beatrix Kocsis^{1,2}, István Molnár^{1,3,4} and Éva Leiter^{1,2}

¹ - Department of Molecular Biotechnology and Microbiology, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

² - HUN-REN-UD Fungal Stress Biology Research Group, Debrecen, Hungary

³ – SW Center for Natural Products Research, The University of Arizona, Tucson, USA

⁴ - VTT Technical Research Centre of Finland, Espoo, Finland

E-mail: pocsi.istvan@science.unideb.hu; ipocsi@gmail.com

Agrobacterium tumefaciens-mediated transformation (ATMT) has been successfully applied in a wide range of basic and applied research projects for the random insertional mutagenesis or targeted genetic modification of both yeasts and filamentous fungi, including important industrial production strains. It is important to highlight that ATMT generally facilitates the efficient integration of transferred DNA into the genome of the target fungus, while eliminating the need for protoplast formation and regeneration: these steps are often inefficient during transformation (Kozák et al. 2018). Over the past decade, we have successfully used this technique for the genetic modification of some ascomycetous fungi, targeting (i) the functional mapping and disruption of the indole-diterpene biosynthetic gene cluster of the hypocrealean ergot fungus *Claviceps paspali* (class *Sordariomycetes*, order *Hypocreales*); (ii) the functional characterization of genes coding for selected elements of the oxidative stress defense system (*BbatfA* and *BbmSOD*) of *Beauveria bassiana* (a hypocrealean entomopathogenic species; class *Sordariomycetes*, order *Hypocreales*); and (iii) the heterologous expression of the *Aspergillus nidulans* *gfdB* gene (encoding a putative glycerol 3-phosphate dehydrogenase) in *A. glaucus* (class *Eurotiomycetes*, order *Eurotiales*),

Kozák et al. (2018) Inactivation of the indole-diterpene biosynthetic gene cluster of *Claviceps paspali* by *Agrobacterium*-mediated gene replacement. *Appl. Microbiol. Biotechnol.* **102**, 3255-3266.

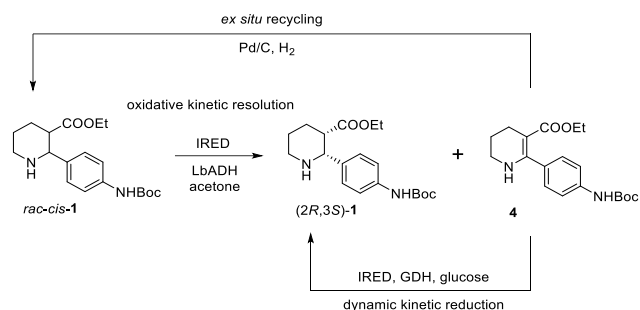
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Innovative redox biocatalysis with double stereocontrol

Zsuzsa Pótáriné Juhász¹, Tihamér Paál¹, Lajos Mészáros¹, Gergely Bánóczy¹, Zoltán Kondor¹, Napsugár Kavalecz¹, Andrea Zsuzsanna Ujvárosi¹, János Végh¹, Dániel Eszenyi¹, Gábor J. Zahuczky¹, Ram Prajapaty², Rushikesh Kadu², Vadivelan Rengasamy², Imre Gyűjtő¹

¹TAPI R&D Hungary – TAPI Hungary Industries Ltd., Debrecen, Hungary, ² TAPI India Pvt. Ltd. - Greater Noida, Uttar Pradesh, India

Biocatalytic production of (2*R*,3*S*)-2-arylpiperidine-3-carboxylate was accomplished by two enantiocomplementary imine reductases (IREDs) through both oxidative and reductive amine-imine transformations. Oxidative kinetic resolution (KR) of *rac*-*cis*-**1** was explored by using imine reductase (IRED) enzymes in an unusual reverse mode of action. The developed process left the desired (2*R*,3*S*)-**1** enantiomer untouched, whereas the undesired one was oxidized and tautomerized *in situ* to enamine **4**. The process was scaled up to kg scale, achieving 99.5% ee and 37.2 g/L/d space-time yield (STY). Furthermore, recycling of **4** by catalytic hydrogenation to *rac*-*cis*-**1** was confirmed at kg scale with 83.7% yield, which resulted in a cumulative yield of 57.8% after one recycling. Repetitive sequences of oxidative KR with ex situ recycling of **4** can give access to 72% overall yield.



The role of genetic factors in the symptom variability of Hungarian tomato brown rugose fruit virus (ToBRFV) isolates

Réka Sárany¹, Asztéria Almási¹, Dóra Pinczés¹, László Palkovics^{2,3}, Katalin Salánki¹

¹ Plant Protection Institute, Centre for Agricultural Research, HUN-REN, Budapest, Hungary, ² Department of Plant Sciences, Albert Kázmér Faculty of Mosonmagyaróvár, Széchenyi István University, Mosonmagyaróvár, Hungary, ³ HUN-REN-SZE PhatoPlant-Lab, Széchenyi István University, Mosonmagyaróvár, Hungary

Since its first report in 2014, *Tobamovirus fructirugosum* (tomato brown rugose fruit virus, ToBRFV) has become a major emerging viral threat across major tomato-growing regions worldwide. Although ToBRFV was reported in Hungary in 2021, no information was available on the molecular properties of the isolates. In this study, ten ToBRFV-infected tomato samples were collected and evaluated under controlled conditions. Comparative symptom development experiments on *Solanum lycopersicum* and *Nicotiana tabacum* cv. Xanthi plants revealed significant differences in both systemic symptoms on tomato and local lesion formation on tobacco. Based on the visual observations three symptom types were established: 1) strong leaf narrowing and smaller plant size on tomato, and large local lesions on tobacco, 2) strong leaf narrowing without differences in plant height on tomato and smaller lesions on tobacco, and 3) mosaic symptoms and smaller lesions. The complete nucleotide sequences of three representative isolates were determined. Comparative sequence analysis identified five non-synonymous mutations among the isolates, four in the RNA-dependent RNA polymerase coding region and one in the movement protein, suggesting the potential connection between the amino acid differences and the symptom diversity. This study provides the first molecular characterization of ToBRFV isolates identified in Hungary.

Antimicrobials and antimicrobial resistant organisms in wastewater

Balázs Seres^{1,2}, Boglárka Pollák¹, Ábel Németh^{1,2}, Eszter Róka¹, Bernadett Khayer¹, Marianna Gáspár¹, Márta Vargha^{1,3}

¹National Center for Public Health and Pharmacy, Department of Public Health Laboratories and Methodology, Budapest, Hungary ²Doctoral School of Environmental Sciences, Eötvös Loránd University, Budapest, Hungary

³One Health Institute, Faculty of Health Sciences, University of Debrecen

The National Centre for Public Health and Pharmacy has recently begun detecting various antibiotic resistance genes and gene families in addition to monitoring upper respiratory pathogens within wastewater-based epidemiological studies. This work follows international trends and new guidelines, as wastewater is a key medium for the environmental spread of resistant and multiresistant microorganisms. The selected targets encode resistance to antibiotics widely used in human medicine and were chosen for their epidemiological relevance. They are detected from DNA isolated from pellets remaining after centrifugation of raw wastewater samples. Primers and probes for RTqPCR were selected from the literature, and copy number controls were established using digital PCR. DNA from bacteria with confirmed resistance served as reference material. Measurements were performed weekly from May 2024 to August 2025, and monthly thereafter, using samples from major city wastewater treatment plants covering 40% of the national population. The parameters can be reliably detected in wastewater, revealing diverse spatial and temporal resistance patterns. Further analysis and comparison with drug consumption and clinical data may help clarify the dynamics of resistance spread and support effective control measures. A pilot shotgun WGS analysis was also conducted to determine the resistance profile of one major city.

Postzygotic evolution of mitogenomes in interspecies *Saccharomyces* hybrids

Matthias Sipiczki, Zsuzsa Antunovics

Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary

Species of the genus *Saccharomyces* are biologically isolated by hybrid sterility. Their hybrids are viable but sterile. Our previous research revealed that two sterility barriers are involved in their biological isolation. Allodiploid hybrids cannot form viable gametes (ascospores) because the chromosomes of the haploid subgenomes cannot pair in meiosis. Genome duplication restores the viability of the spores (gametes) but not their fertility because their mating programme is repressed by mating-type heterozygosity. Observations made in other laboratories have raised the possibility that nucleo-cytoplasmic incompatibilities may also contribute to hybrid sterility. Our recent results did not confirm this view and demonstrated that mitogenomes are not involved. Examination of a large number of „cevarum” (*S. cerevisiae* x *S. uvarum*) hybrids revealed that the allopolyploid nuclear genome is compatible with both parental mitogenomes and also with their recombined versions. During mating, the *S. cerevisiae* mitogenome was preferentially transmitted to the hybrids, but more than one third of the hybrids had chimeric (recombinant) mitogenomes. The high frequency of recombination indicates that allospecific mitochondria can fuse and their DNAs can interact. We identified 34 recombination sites, almost exclusively in coding regions. The chimeric mitogenomes had diverse combinations of parental and recombined mitochondrial genes, and all combinations were compatible with the hybrid nuclear genome. Certain recombination sites coincided with homing sites of the *S. cerevisiae* mitogenome. Recombination did not corrupt either nucleo-mitochondrial compatibility or the mitochondrial function, but destabilised the mitogenome, resulting in respiration-deficient (petite) segregants. The loss of respiration proficiency was associated with rearrangements and size reduction in the mitogenome of the hybrid. Since the loss of the ability to respire impairs fitness, interspecies hybrids that have recombined mitotypes are less competitive in mixed populations with parental strains.

Bacterial and fungal DNA profiles in patients with brain tumors: a multi-compartment pilot study

László Sipos¹, Péter Banczerowski¹, János Juhász^{2,3}, Imre Fedorcsák¹, György Berényi¹, Nóra Makra², Zsuzsanna A. Dunai^{2,4}, Loránd Erőss¹, Dóra Szabó^{1,2,4},

¹ Department of Neurosurgery and Neurointervention, Semmelweis University, 1085 Budapest, Hungary; ² Institute of Medical Microbiology, Semmelweis University, 1089 Budapest, Hungary; ³ Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, 1083 Budapest, Hungary; ⁴ HUN-REN-SU Human Microbiota Research Group, 1052 Budapest, Hungary

Background and objective: Bacterial and fungal DNA profiles have been described in several cancer types, but data on brain tumors remain limited. We aimed to compare bacterial and fungal DNA profiles across gut, blood, tumor-adjacent tissue, and tumor tissue samples.

Methods: This prospective pilot study included five patients undergoing surgery for brain tumors: two with glioblastoma, one with anaplastic meningioma, and one each with a brain metastasis from breast or hepatic cancer. Tumor tissue, tumor-adjacent tissue (TAT), whole blood, and anorectal swab samples were analyzed from each patient. Blood and gut samples were collected before surgery and perioperative antibiotic prophylaxis. Bacterial DNA was analyzed by amplicon sequencing of the V3-V4 region of the 16S rRNA gene, and fungal DNA by ITS amplicon sequencing on the Illumina MiSeq platform. Extraction-negative, PCR-negative, and positive controls were included in each run. Alpha diversity was assessed using the Shannon index and beta diversity using the Jaccard method.

Results: Bacterial alpha diversity calculated from the DNA profiles was highest in gut samples and was significantly greater than in tumor ($p = 0.009$), TAT ($p = 0.014$), and blood samples ($p = 0.009$). Based on beta diversity, the bacterial DNA profiles of gut samples also separated from those of the other sample types. In contrast, within individual patients, the bacterial DNA profiles of blood, TAT, and tumor tissue were highly similar and were dominated by *Enterococcus*, *Klebsiella*, *Blautia*, and *Lactobacillus*. No significant differences were observed in the alpha or beta diversity of fungal DNA profiles; however, their composition showed marked interindividual and sample-type-specific variation. The fungal DNA profiles of blood and TAT were similar in four patients, whereas the tumor profile clearly differed from the blood and TAT profiles in four cases. Sequences assigned to *Tomentella*, *Alternaria*, and *Didymosphaeria* were frequently detected in blood and TAT, while tumor tissues contained sequences including *Alternaria*, *Malassezia*, *Schizophyllum*, and *Tomentella*.

Conclusion: Our findings suggest a relationship between the bacterial DNA profiles of blood, the tumor microenvironment, and tumor tissue, whereas tumor-associated fungal DNA profiles may show distinct, patient-specific features. These results from a small cohort are hypothesis-generating. Larger, standardized studies including control groups are required to evaluate the potential biomarker value of microbial DNA detected in blood.

From mycotoxin production to antibiotic potentiation: Exploring the bioactive potential of beauvericin, an emerging mycotoxin

Suhajda Ákos¹, Háhn Judit², Kaszab Edit², Sörös Csilla³, Farkas Milán¹, Tánicsics András¹, Kriszt Balázs², Cserháti Mátyás¹

¹ Department of Molecular Ecology, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences (MATE), Páter Károly utca 1, 2100 Gödöllő, Hungary, ² Department of Environmental Safety, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences (MATE), Páter Károly utca 1, 2100, Gödöllő, Hungary ³ Department of Food Chemistry and Analysis, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences (MATE), Villányi út. 29-43, H-1118 Budapest, Hungary

Beauvericin (BEA) is one of the most frequently detected, emerging mycotoxin exhibiting a broad range of biological activities, including antimicrobial and insecticidal effects. Despite its widespread occurrence, BEA is not under food safety regulation. According to the EFSA (2024) the available data on its toxicity indicate that BEA has no genotoxic potential, and possibly not pose as significant risk for human or animal health.

In the first part of this study, the BEA-producing ability of 100 *Fusarium* isolates collected in Hungary was investigated. PCR based screening targeting the beauvericin synthetase (BEAS) gene identified the corresponding gene region in 23 out of 100 isolates, followed by UHPLC–ESI–MS/MS analysis to verify BEA production. *Fusarium proliferatum* and *F. verticillioides* isolates exhibited the highest BEA-producing capacities, with a maximum concentration of 3131 mg/kg. To the best of the author's knowledge, these investigations provided the first evidence of BEA biosynthesis by *F. verticillioides*, extending the known spectrum of BEA-producing *Fusarium* species.

Beyond its significance as a fungal contaminant, BEA's efficacy has been demonstrated in the literature against wide range of Gram-negative and Gram-positive bacteria, and also a potentiating effect when it combined with other compounds. The interaction of BEA and a last-resort antibiotic, colistin (COL) was tested on 7 different multi-resistant *P. aeruginosa* isolates by microplate assay. Although, BEA exhibited no antibacterial effect on the investigated isolates up to the highest tested concentration (50 µg/mL), BEA at non-inhibitory concentrations (2.5–10 µg/mL) significantly enhanced the antibacterial activity of COL (1 and 2 µg/mL) in six out of seven isolates, resulting in a marked reduction in residual bacterial growth.

Consequently, BEA may open up new opportunities for the treatment of clinically significant multidrug-resistant *P. aeruginosa* infections as it can potentiate the bactericidal effect of COL. Our results provide the first insight into the potentiating effect of BEA in a mixture with a medically important last resort antibiotic against critically important opportunistic *P. aeruginosa* isolates.

These observations further demonstrate that BEA represents not only an agriculturally relevant fungal contaminant, but also a bioactive natural product whose interactions with bacterial cells and antimicrobial agents warrant further mechanistic investigation.

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Development of isothermal amplification diagnostic methods for the rapid detection of economically significant sturgeon viruses (alloherpesvirus, mimivirus)

András Surján, Andor Doszpoly

HUN-REN Veterinary Medical Research Institute, Budapest, Hungary

Objective: Aquaculture has a growing demand for rapid, highly sensitive diagnostic methods that can be applied in the field without laboratory facilities. Our research aimed to develop and validate isothermal amplification systems (RPA and LAMP) suitable for detecting the Siberian sturgeon herpesvirus (SbSHV) and the Acipenser Iridovirus-European (AcIV-E), which could later form the basis of a complete CRISPR-Dx system.

Methods: For the detection of SbSHV, primers were designed for the RPA and LAMP methods based on the sequence of the DNA polymerase and ATPase subunit of terminase genes. In the case of AcIV-E, a LAMP procedure was optimised targeting the hypothetical nvc 153 gene. The performance of the methods was tested and validated using virus isolates serving as positive controls, sturgeon cell line, dilution series, and incoming samples. Previously optimised qPCR measurements were used as a reference. The detection of LAMP amplification for both viruses was evaluated using a real-time fluorescent method (qPCR), gel electrophoresis, and a visual, colorimetric LAMP method. The specificity of the obtained amplicons was also validated by Sanger sequencing for every positive result.

Results: During analytical examinations on samples originating from cell culture supernatants, the LAMP methods proved to be functional for both viruses (SbSHV and AcIV-E). Measurements performed on dilution series confirmed that the sensitivity of the LAMP assays reaches that of the qPCR reference, reliably detecting targets even up to a 10^8 -fold dilution level. The colorimetric LAMP procedure was successful for both pathogens, providing clear, naked-eye visible, instrument-independent results in the case of all tested samples. In contrast, the sensitivity of the RPA test optimized for SbSHV fell significantly short of this, showing a specific signal only up to a 10^2 -fold dilution. Sanger sequencing unambiguously confirmed the specificity of the obtained LAMP results in all cases, and ruled out the cross-contamination and false-positive results characteristic of the method.

Conclusion: The sensitivity and robustness of the developed LAMP systems approach those of traditional qPCR procedures, thus providing a good foundation for field diagnostics or a future CRISPR-based platform. The final validation of the method's specificity is currently ongoing using healthy and infected sturgeon samples originating from domestic fish farms.

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Characterisation of lytic bacteriophages isolated from free ranging brown rats (*Rattus norvegicus*) showing activity against enterobacteria

Domonkos Sváb¹, Réka Sipos¹, Ama Szmolka¹, László Egyed¹

¹ Enteric bacteriology and alimentary zoonosis research group, HUN-REN Veterinary Medical Research Institute, Budapest, Hungary

Wild rodents serve as reservoirs of diverse bacterial pathogens and potentially, their bacteriophages. Our study aimed to isolate and characterize bacteriophages infecting *Escherichia coli* K-12 MG1655, enterohemorrhagic *E. coli* (EHEC) O157:H7 Sakai and *Citrobacter rodentium* from the intestinal content of free-ranging brown rats (*Rattus norvegicus*) captured in Hungary. Thirteen phages were isolated and characterised phenotypically and genomically. Phylogenetic analysis showed that the phages belonged to genera *Tequatrovirus*, *Vequintavirus*, *Felixounavirus*, and *Hanrivervirus*. Six phages represented a new species within the *Vequintavirus* genus and showed approx. 100% sequence identity despite being isolated from six different rat samples across the country. Three phages represented two novel *Felixounavirus* species, while the three *Hanrivervirus* isolates defined two new species within that genus as well. The one *Tequatrovirus* isolate also represented a new species within the genus. Genome analysis showed the lack of enterobacterial virulence or resistance determinants in all phages and indicated their strictly lytic lifestyle as well. Thermal and pH-stability tests showed that the phages preserved their activity after prolonged (24 h) exposure to room temperature, and similarly tolerated pH values of 5 and 9 as well. In contrast, an incubation for 1 hour at 80 °C, or at pH 1 or 13 caused complete loss of activity when compared to phage stocks stored at 4 °C in a - medium with neutral pH. This is the first study to report lytic phages isolated from free-ranging rats, capable of lysing enterobacterial pathogens. Our findings also highlight the potential of rats as a source for phages with biocontrol or therapeutic applications.

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Microbial interactions promoting the pathogenesis of Esca disease in grapevine

Dóra Szabó¹, Nikolett Molnár^{1,2}, Ádám Novák¹, Adrienn Gomba-Tóth¹, Kálmán Zoltán Váczy¹, Zoltán Karácsony¹

¹Eszterházy Károly Catholic University, Food and Wine Research Institute, Leányka utca 8., Eger, H3300, Hungary; ²Hungarian University of Agricultural and Life Sciences, Doctoral School of Natural Sciences, Páter Károly u. 1, Gödöllő, H2100, Hungary

Esca is an important, yet poorly understood fungal disease of grapevine. Several wood-colonizing fungi are associated with this syndrome, leading to the development of chlorosis and necrosis on berries and eventually premature host death. Among the associated pathogens, one of the most relevant is the ascomycetous species *Phaeomoniella chlamydospora* (Pch), which is considered a pioneer pathogen. While the pathogenesis of Esca -because of its unusual characteristics- is still enigmatic, a growing body of evidence suggests that microbial interactions involving fungi and even bacteria may be crucial for Esca expression. Our investigations examining the interactions between Pch and grapevine endophytic microbes revealed the putative positive role of *Aureobasidium pullulans* (Apu) fungus and *Erwinia billingiae* (Ebi) bacterium in Esca expression through interactions with Pch. The abundance of Apu showed a positive correlation with Esca foliar symptom expression in the field, and the fungus modulated Pch development in vitro. Ebi and Pch showed mutual growth modulation in mixed cultures in a carbon source-dependent manner, resulting in growth enhancement in the presence of saccharose, and growth inhibition in the case of pectin. Investigating the expression of virulence-related traits in Pch showed that Ebi promotes the secretion of the phytotoxic polysaccharide pullulan by Pch, and enhances the ability of the fungus to degrade phenolics. In planta examinations showed that both Apu and Ebi promote the development of foliar damage in Pch-affected grapevine leaves.

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Targeted LC-MS analysis of non-proteinogenic amino acids and oligopeptides induced in mouse testes by the lactic acid bacterium *Lactiplantibacillus plantarum* SNI3, and non-targeted LC-MS analysis of metabolites produced by SNI3

Helga Szalontai¹, Zoltán Szilágyi¹, Miklós Lovas¹, Marietta Korózs¹, Ildikó Vidács¹, Judit Kosztik¹, János Barna^{1,2}, István Nagy¹, József Kukolya¹

¹ Food and Wine Research Institute, Eszterházy Károly Catholic University, Eger, Hungary, ² HUN-REN-ELTE Genetics Research Group, Department of Genetics, Eötvös Loránd University, Budapest, Hungary

The aim of our research is to develop a reprobiotic screening system that can identify metabolomic and transcriptomic markers useful for evaluating strains of the former genus *Lactobacillus* with positive effects on male fertility. The work investigates the gut microbiome–reproductive system connection.

According to our previous results [1], four-week administration of the *Lactiplantibacillus plantarum* SNI3 (SNI3+) strain significantly increases testosterone levels, sperm count, testis size and sexual activity in mice. Metabolome analysis of the testis identified numerous up- and down-regulated metabolites. Non-proteinogenic amino acids (NPAAs) and peptides (NPPs) up-regulated in the testes of SNI3-treated male mice included dimethylglycine (DMG), γ -aminobutyric acid (GABA), R- β -aminoisobutyric acid (BAIBA), γ -glutamyl dipeptides (γ -Glu-Glu and γ -Glu-Gln) and oxidised glutathione (GSSG).

In the present study we performed a comparative analytical investigation with two main objectives: (1) to determine the optimal sample-preparation method that enables efficient metabolome profiling of large numbers of biological samples; and (2) to compare the SNI3+ strain with its plasmid-cured derivative (SNI3-) in order to assess how plasmid loss affects the metabolite profile. Both targeted (focused on the above-mentioned up-regulated metabolites) and non-targeted LC-MS profiling were applied. Measurements were carried out with an LC QTOF MS system using a Poroshell 120 HILIC-Z (150 x 2.1 mm; 2.7 μ m) column and electrospray ionization in negative mode.

Our results show that liquid MRS (de Man-Rogosa-Sharpe) medium is unsuitable for targeted and non-targeted LC-MS analyses because its complex composition masks the compounds of interest and may contain metabolites secreted by lactobacilli. In contrast, cultivation on solid MRS plates proved suitable. The LC-MS datasets were analysed with Mass Hunter Profinder for finding molecular features across sample groups. This approach proposed several peptide-like biomolecules in SNI3+ samples; their identification is still in progress.

In subsequent steps we will examine the effect of the SNI3 strain (and its metabolites) on Caco-2 cells modelling the absorptive epithelium of the small intestine, and the biological effects of the metabolites up-regulated in mouse testes. We have also included the *Caenorhabditis elegans* model system to investigate broader biological effects.

[1] Juhász, B., Horváth, K., Kuti, D. *et al.* Dipeptide metabolite, glutamyl-glutamate mediates microbe-host interaction to boost spermatogenesis. *Sci Rep* 14, 21864 (2024).

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Characterization of beneficial bacterial strains from drought-affected regions of Hungary

Viktor Szentpéteri¹, Ákos Juhász¹, Zoltán Mayer¹, Livia László¹, Katalin Posta¹

¹ Department of Microbiology and Applied Biotechnology, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

The increasing frequency of drought events poses significant challenges to agricultural productivity, highlighting the need for sustainable strategies to improve crop resilience. Despite extensive research on plant growth-promoting (PGP) microorganisms, there remains a critical need to identify and characterize novel indigenous strains that combine multiple beneficial traits with robust tolerance to abiotic stress, thereby providing promising candidates for further agricultural use.

In this study 90 microbial isolates from 16 sampling sites were obtained from the heavily drought affected areas of Hungary, mainly the Hungarian Great Plain. The isolates were screened for several PGP properties. Phosphate (P) solubilization was assessed on Pikovskaya agar, nitrogen (N) fixation was evaluated using Burk's nitrogen-free medium, indole-3-acetic acid (IAA) production was quantitatively determined using tryptophan-supplemented medium with Salkowski colorimetric assays, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity was investigated using Dworkin and Foster minimal medium supplemented with ACC as the sole N source, and lastly biocontrol capabilities were screened against plant pathogen fungi. Furthermore, the presence of the phosphatase (*phoD*) and ACC deaminase (*acdS*) genes was confirmed by PCR. Ten isolates exhibiting multiple PGP traits were identified via 16S rRNA gene sequencing, with the majority belonging to the genus *Pseudomonas*. The osmotic stress tolerance through the cultivation in the presence of PEG 6000 and pH resilience across three different pH levels of the identified strains was also studied in order to select the most versatile regarding their survivability and biostimulant capabilities.

Among the isolates, isolate 1379 (*Pseudomonas vancouverensis*) was particularly noteworthy as it has shown the ability to perform all of the examined PGP properties. Moreover 1379 also demonstrated strong growth on all PEG concentrations and pH levels indicating good drought resilience and also a broad pH tolerance.

This research was supported by the Flagship Research Groups Programme of the Hungarian University of Agriculture and Life Sciences; and the EKÖP-MATE/2025/26/K university research Scholarship Programme of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund.

Comparison of JCV-Specific IgG seroprevalence between healthy individuals and patients in Hungary

Katalin Réka Tarcsai¹, Adrienne Lukács¹, Erzsébet Barcsay¹, Katalin Szomor¹

¹ Department of Microbiological Reference Laboratories, National Center for Public Health and Pharmacy, Budapest, Hungary

John Cunningham virus (JCV) is a human polyomavirus of the Polyomaviridae family that typically persists asymptomatically in healthy individuals. However, JCV can be reactivated during immunosuppression or certain immunomodulatory treatments, such as natalizumab used in multiple sclerosis (MS), and may lead to the development of progressive multifocal leukoencephalopathy (PML). Therefore, determining JCV infection and monitoring serological status are important for assessing the risk of PML development.

The aim of our research was to compare the seroprevalence of anti-JCV IgG antibodies between healthy individuals and patients and to examine the correlation between PCR and serological results in patients for whom an appropriate sample type was available.

The presence of JCV-specific IgG antibodies in serum samples was determined using a validated ELISA assay with high sensitivity and specificity (VIDITEST anti-JCV IgG; Videa), according to the manufacturer's instructions. In addition, real-time PCR was performed using GeneProof™ BK/JC Virus PCR Kit on serum samples from nearly 20% of the patients. Where available, other clinical sample types, including cerebrospinal fluid and urine, were additionally tested by real-time PCR.

Approximately 60% of the samples tested were JCV seropositive. In both populations, an increasing seropositivity rate was observed with increasing age, with the highest prevalence in the age group over 40 years. The median antibody concentration was higher among healthy individuals. In the patient group, the majority of the tested cases were individuals diagnosed with MS or showing clinical symptoms suggestive of the disease. However, JCV-seropositive cases were predominantly identified among patients undergoing pre-treatment screening and those presenting with other symptoms. In the PCR analysis, most positive results were detected in urine samples, while only a small number of positive results were observed in cerebrospinal fluid and serum samples.

Our results confirm that JCV antibody positivity is high in the Hungarian population, consistent with data from the international literature. The more moderate antibody response observed in the patient group highlights the importance of structured, regular serological screening and continuous monitoring both before and during high-risk biological treatments to facilitate PML risk assessment.

These findings are of particular clinical relevance for patients with symptomatic MS, for whom accurate risk assessment is essential to mitigate the risk of PML and support safe and informed therapeutic decision-making. The number of samples tested by PCR was limited; therefore, further conclusions should be drawn with caution. Nevertheless, our results suggest that simultaneous submission of multiple sample types for PCR testing may improve the reliability of monitoring viral reactivation.

A simple precipitation-based method for peptaibol purification from *Trichoderma*

Gergő Terna^{1,2}, Fanni Kovács^{1,2}, Dóra Balázs¹, Henrietta Hunkár^{1,2}, Ákos Rozsnyói^{1,2}, Mónika Varga¹, András Szekeres¹, Isidora Knežević³, Helena Todorović³, Jovan Lazarević³, Gordana Racić³, Csaba Vágvolgyi¹, Tamás Papp¹, Chetna Tyagi¹, László Kredics¹, Tamás Marik¹

¹ Department of Biotechnology and Microbiology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary, ² Doctoral School of Biology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary, ³ Tamiš Research and Development Institute, Novoseljanski put 33, 26000 Pančevo, Serbia

Peptaibols are highly diverse, promising antimicrobial peptides, mostly produced by the soil dwelling filamentous fungal genus *Trichoderma*. These peptides contain an amino-alcohol group at the C-terminus, and an acetylated N-terminus. Non-proteinogenic amino acids, such as α -aminoisobutyric acid and D-isovaline are incorporated into their 6-20 long amino acid sequences. Owing to these properties, peptaibols can form voltage-gated ion channels in the biological membranes which can lead to their antimicrobial effect. Peptaibols are naturally synthesized by non-ribosomal peptide synthetases (NRPSs) which accounts for the high diversity in their amino acid sequences.

The aim of our research group is to identify, purify and characterize the peptaibols produced by various *Trichoderma* strains. To achieve this, we incubate the selected fungal strains on malt extract media for 7 days and the peptaibols are extracted by the chloroform:methanol 2:1 v/v solvent mixture. To identify the peptaibols and determinate their amino acid sequence we utilize High-Performance Liquid Chromatography-High Resolution Mass Spectrometry (HPLC-HRMS). The promising strains are then selected for large scale cultivation on large glass trays with malt extract media. The peptaibols are extracted by the chloroform:methanol (2:1, v/v) solvent mixture. This extract is evaporated onto silica and purified with flash chromatography resulting in 60-70 fractions. Each sample is adjusted to a 10 mg/ml concentration and screened against *Micrococcus luteus* to detect the biological activity of the peptaibol containing fractions. However, a major limitation of this procedure is its extensive time and solvent consumption.

Therefore, this study aims to develop a simple, cost-effective method for peptaibol purification. The peptaibols from *Trichoderma longibrachiatum* f. *bisettii* SZMC 12546 were successfully precipitated using organic solvents and salts. The purity and biological activity obtained with this novel approach were comparable to those achieved by flash chromatography. Nevertheless, peptaibols exhibit high structural diversity with each *Trichoderma* strain producing a unique peptaibol profile characterised by distinct properties. While the peptaibols from *T. longibrachiatum* f. *bisettii* SZMC 12546 were successfully purified with this new protocol, the same procedure proved unsuitable for *T. reesei* SZMC 22616 peptaibols. Currently, we are optimizing the method by evaluating different salt concentrations, and by varying temperatures across several organic solvents. A universally successful and cost-effective purification process for peptaibols could make the practical use of peptaibols more accessible in both agricultural and biomedical applications.

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Air Quality in Primary School Classrooms – Microscopic Fungi as Biological Air Pollutants

Zsófia Tischner¹, Tamás Szigeti², Edit Kaszab¹, Balázs Kriszt¹, Donát Magyar²

¹ Department of Environmental Safety, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary, ² National Center of Public Health, Budapest, Hungary

Background & Aims

Indoor air quality is an important determinant of human health, as people spend approximately 80–90% of their time indoors. Long exposure to unsatisfactory indoor air quality could easily lead to 'building-related illnesses' (BRI), such as respiratory illnesses, allergy or asthma. Children, one of the most sensitive age groups, spend approximately 6-8 hours in school on weekdays. This study aimed to investigate airborne fungal contamination in Hungarian primary school classrooms.

Methods

Air sampling was undertaken with a single stage Andersen sampler (MAS100) from 34 classrooms of 22 elementary schools in Hungary in Sinphonie (2011-2012, 18 classrooms) and InAirQ (2017-2018, 16 classrooms) projects. The samples were taken during the lessons with closed windows and doors. Outdoor samples were also taken from the schoolyards as references. To detect fungi, air samples were incubated on malt extract agar with 2% chloramphenicol at 25 °C for five days. Concentrations (C) were expressed in colony forming units (CFU/m³). Threshold level was defined as indoor C of a given fungal morphotype > outdoor C of a given fungal morphotype +50 CFU. Sporulating filamentous fungi were identified at genus level, with a Carl Zeiss Jenaval light microscope at 300× magnification. Isolates of filamentous fungi exceeding threshold levels were identified by the ITS (ITS1-5.8S- ITS4) regions of the rDNA repeats.

Results

A total of 28 fungal genera were identified in indoor air. The highest total concentrations were measured of the following indoor fungi: *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Acremonium*. In 16 classrooms, one or more fungal taxa exceeded the recommended indoor/outdoor criterion, consistent with the presence of indoor fungal growth. The taxa exceeding the threshold level were: *Acremonium* sp., *Alternaria* spp., *Aspergillus* sect. *Versicolores*, *Cladosporium* spp., *Geotrichum* sp., *Penicillium digitatum*, *Penicillium* spp. Four schools were highly contaminated with opportunistic pathogenic and/or allergenic microscopic fungi (*A. sect. Versicolores*, *P. digitatum* and *Penicillium* spp.).

Conclusion

The results emphasise the importance of the prompt detection and remediation of moisture damage and mould contamination in school buildings, to safeguard children's health. The present survey, provides baseline data on airborne fungal contamination in Hungarian primary schools. Additional sampling campaigns are planned in 2026.

Development of recombinant plant defensin-based biofungicides for sustainable crop protection

Liliána Tóth¹, Rebeka Papp^{1,2}, László Galgóczy¹

¹ Department of Biotechnology and Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary,
² Doctoral School of Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

The increasing incidence of fungicide resistance together with the progressive withdrawal of conventional fungicides has created an urgent need for environmentally friendly alternatives in crop protection. Plant defensins represent promising biofungicide candidates owing to their potent antifungal activity, favorable safety profile and biodegradability. In the present study, we investigated the applicability of two recombinant tomato-derived defensins (K4CBP6 and B1N680) in novel spray formulations designed for sustainable plant protection.

The antifungal activity of the defensins was evaluated against both wild-type and fungicide-resistant *Botrytis cinerea* isolates. In addition, the effects of two commercial adjuvants (Heliosol and Permetfix) were investigated alone and in combination with the defensins. Separately, combinations of Heliosol with currently used commercial fungicides representing different modes of action, including Cantus (boscalid), Cuproxat (tribasic copper sulfate), Dagonis (fluxapyroxad + difenoconazole), fluopyram, and Teldor (fenhexamid), were evaluated.

Both defensins efficiently inhibited the growth of wild-type and fungicide-resistant *B. cinerea* isolates, exhibiting minimum inhibitory concentration (MIC) values of 12.5–25 µg/mL. Heliosol displayed intrinsic antifungal activity, whereas Permetfix showed no inhibitory effect and occasionally promoted fungal growth. Combination experiments demonstrated that Heliosol preserved, and in several cases enhanced, the antifungal efficacy of the defensins. Among the tested commercial fungicides, all except Cantus inhibited fungal growth, frequently at concentrations below the recommended field dose. While Heliosol generally produced little or no improvement when combined with most fungicides, its combination with Cantus resulted in a marked increase in antifungal activity, suggesting a substantial enhancement of formulation efficacy.

These findings demonstrate that recombinant plant defensins remain highly active against fungicide-resistant plant pathogens and highlight the importance of formulation strategies in maximizing their practical performance. These results support the further development of defensin-based biofungicides as sustainable alternatives or complements to conventional chemical fungicides.

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Enhancing stress tolerance in grain sorghum (*Sorghum bicolor*) using PGP bacterial Isolates

Máté Tóth¹, Renáta Ábrahám¹, Ines Amara¹, Ákos Suhajda¹, Emma Klára Bajzák¹, Erzsébet Baka¹, Mátyás Cserhádi¹, András Táncsics¹, Balázs Kriszt¹, Julianna Kobolák¹, Ana Fita³, Francesca Mapelli⁴, Milán Farkas¹

¹ Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

² Department of Environmental Safety, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary.

³ Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universitat Politècnica de València, Spain

⁴ Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Milan, Italy

Climate change is posing increasing challenges to agricultural production, highlighting the need for innovative approaches to enhance crop resilience. Increasing drought stress is making the cultivation of maize and other forage crops economically less viable, while sorghum (*Sorghum bicolor*) represents a promising alternative due to its lower water and nutrient requirements. The RhiSorgh4Health project is an international collaboration between the Hungarian University of Agriculture and Life Sciences (MATE), the Universitat Politècnica de València (UPV, Spain), and the University of Milan (UNIMI, Italy). One of the key objectives of the project is to further enhance the drought tolerance and production efficiency of sorghum through the application of plant growth-promoting (PGP) bacterial strains.

Bacterial strains were isolated from the root surfaces and internal root tissues of naturally drought-adapted plants (*Elymus repens*, *Poa annua*, *Festuca rupicola*, *Helictotrichon pubescens*, and *Koeleria* sp.) collected from a dry grassland in Hungary. Reasoner's 2A (R2A) agar and Nitrogen-Free Bromothymol blue (NFB) medium were used for isolation. In total, 286 rhizosphere and endophytic bacterial isolates were obtained. Identification was performed using MALDI-TOF mass spectrometry and 16S rRNA gene sequencing.

Most isolates belonged to the genera *Bacillus*, *Flavobacterium*, *Paenarthrobacter*, *Pantoea*, *Pseudomonas*, and *Streptomyces*, although the identification of several isolates is still ongoing. The PGP potential of the isolates will be assessed based on nitrogen fixation, indole-3-acetic acid (IAA) production, phosphate mobilization, siderophore and exopolysaccharide (EPS) production, and ACC deaminase activity.

The 15 most promising strains will subsequently be evaluated for their effects on sorghum plantlets in short-term sterile phytotron experiments in collaboration with UPV. At UNIMI, selected strains will also be chromosomally tagged with genes encoding fluorescent proteins, enabling visualization of their root colonization patterns.

The research was conducted within the framework of the project 2025-1.2.1-HU-RIZONT-2025-00110, entitled "RhiSorgh4Health - A Sorghum microbiome project – from climate resilience to the One Health perspective

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The genomic epidemiology of *Candida parapsilosis* bloodstream isolates collected between 2013 and 2025 at a tertiary care center

Zoltán Tóth^{1,2}, Andrea Harmath^{1,3}, Zsófia Rádi¹, Bálint Farkas^{1,2,3}, Ágnes Jakab^{1,2}, László Majoros^{1,2}, Renátó Kovács^{1,2}

¹ Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary ² Medical Microbiology, University of Debrecen, Debrecen, Hungary ³ Doctoral School of Pharmaceutical Sciences, University of Debrecen, Debrecen, Hungary

Fluconazole-resistant *Candida parapsilosis* outbreaks are well documented. Much less is known however about the genomic epidemiology of this opportunistic pathogen in the absence of antifungal resistance.

In this study we have evaluated 57 *Candida parapsilosis* bloodstream isolates collected between 2013 and 2025 at the University of Debrecen Clinical Centre. Isolates were sequenced on the Illumina NextSeq platform. Phenotypic susceptibility testing of the isolates was performed according to CLSI M27 ed.3, while biofilm-forming ability was assessed using both Crystal-Violet and XTT assays. Furthermore 30 days mortality and spatial distribution of the isolates between hospital wards were also examined.

We observed significant clustering of biofilm-forming isolates within one closely related clade spanning 13 years, with a maximum SNP distance of 350, although most isolates within the clade fell within 100 SNPs. A chi-square test with Monte Carlo simulation indicated that isolate distribution across hospital wards was non-random; however, a probable common source could not be identified. Linear regression revealed no significant trend of increasing biofilm mass over time, by either the CV or XTT assay. Neither crude mortality nor time to death differed significantly between the dominant clade and unrelated isolates. Antifungal resistance was absent in all isolates.

Our results suggest that *Candida parapsilosis* hospital population may have endemic clades and biofilm forming ability may be associated with long-term hospital persistence of a given lineage even in the absence of acquired resistance mechanisms.

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Characterization of biofilm formation and biofilm-associated antifungal drug resistance of *Malassezia pachydermatis* strains isolated from dogs

Zsófia Turóczy¹, Krisztina Pintér¹, Enikő Wehmann¹, Tibor Magyar¹, Marianna Domán¹

¹HUN-REN Veterinary Medical Research Institute, Budapest, Hungary

M. pachydermatis is part of the normal microbiota of the skin and mucous membranes of numerous warm-blooded animals, where it typically persists as a commensal organism. It is commonly isolated from the lipid-rich areas of the skin and mucous membranes of dogs and cats, which provide favourable conditions for its growth. However, the alteration of certain environmental conditions (elevated humidity, immunodeficiency) may disturb the established balance between the fungus and the host. Under such conditions, *M. pachydermatis* can proliferate excessively and act as an opportunistic pathogen. The most frequent diseases associated with its overgrowth are otitis externa (inflammation of the external ear canal) and dermatitis (inflammation of the skin). *M. pachydermatis* can form biofilms that are structured microbial communities embedded in an extracellular matrix. Biofilm formation may promote fungal persistence and reduce susceptibility to antifungal therapy by limiting drug penetration. To date, only a few data are available regarding the extent of biofilm formation of *M. pachydermatis* and the antifungal drug resistance of *M. pachydermatis* biofilms. The aim of our study was to evaluate the biofilm-forming ability of 83 *M. pachydermatis* strains using crystal violet microtiter plate assay. Based on the obtained absorbance values, the isolates were categorized as strong, moderate, weak, or non-biofilm producers. Of the 83 strains examined, 19 (22.9%) were classified as strong biofilm producers, 35 (42.2%) as moderate producers, 26 (31.3%) as weak producers, and 3 (3.6%) as non-biofilm producers. In a previous study, the minimum inhibitory concentrations (MICs) of ketoconazole, terbinafine, and itraconazole were determined against planktonic *M. pachydermatis* cells. To further investigate the potential relationship between biofilm-forming capacity and antifungal susceptibility, *in vitro* antifungal susceptibility testing was carried out with the isolates classified as strong or moderate biofilm producers after biofilm formation. Compared with the corresponding MICs determined for planktonic cells, higher MIC values were observed for 43 of 54 strains exposed to ketoconazole (79.6%), 33 of 54 strains exposed to itraconazole (61.1%), and 46 of 54 strains exposed to terbinafine (85.2%). Our results support that biofilm formation may substantially impair antifungal activity against *M. pachydermatis* in an isolate-dependent manner, potentially due to the biofilm matrix or the altered metabolic activity of biofilm-associated cells. Our future plans include studying the metabolic changes associated with biofilm development by comparing the metabolic profiles of *M. pachydermatis* cells before and after biofilm formation. These studies may provide further insight into the molecular mechanisms underlying biofilm development and identify potential targets for strategies aimed at preventing or disrupting biofilm-associated infections.

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The microbiologist's modern toolbox

Andrea Zsuzsanna Ujvárosi¹, Boglárka Szabóné Szikszai¹

¹ TAPI Hungary Industries Ltd., Debrecen, Hungary

In recent years, microbial biotechnology has undergone a technological transformation, with the emergence of advanced analytical platforms and real-time monitoring systems that significantly enhance experimental precision and throughput. The presentation provides an overview of modern tools that are reshaping the microbiologist's workflow—from early screening to fermentation optimization.

We will explore the application of the Omnilog Phenotype MicroArray, a high-throughput platform enabling detailed phenotypic profiling of microorganisms based on their metabolic and stress response capabilities. This tool is invaluable for strain characterization, metabolic pathway analysis, and adaptive evolution studies.

Next, we present the MPS (Multi Parameter Sensor) system, which allows real-time, non-invasive monitoring of key fermentation parameters such as biomass, pH, DO, and oxygen transfer rate directly in shaken flasks. This technology bridges the gap between small-scale screenings and scalable process development. An alternative is the PreSens optical sensor technology that enables accurate measurement of dissolved oxygen and pH in shake flasks and microtiter plates.

Finally, the Aquila LIS (Liquid Injection System) is introduced as an innovative tool for precise, programmable addition of liquids directly into shake flasks. This capability supports fed-batch experiments and dynamic condition control during early-stage cultivation, allowing for more realistic process simulations and kinetic studies at lab scale.

In the presentation, we provide an insight into the world of an industrial microbiologist's life utilizing the aforementioned toolbox.

The versatile role of heat shock factor 1 HSF-1 in the immunity of the nematode *Caenorhabditis elegans*

Viktor Vázsony Vincze¹, Márton Kovács¹, Bernadette Hotzi¹, Tímea Sigmond¹, Judit Kosztik³, Tibor Vellai^{1,2}, János Barna^{1,2,3}

¹Department of Genetics, Eötvös Loránd University, Budapest, Hungary ²Genetics Research Group, HUN-REN-ELTE, Budapest, Hungary ³Food and Wine Research Institute, Eszterházy Károly Catholic University, Eger, Hungary

The heat shock factor HSF1 is an evolutionarily conserved transcription factor that, in addition to its function in the cellular stress response, plays an important role in fundamental biological processes such as development, metabolism and aging, as well as in certain diseases, including neurodegenerative disorders and cancer. A role for HSF1 in immunity has been demonstrated in both vertebrates and invertebrates. In the nematode *Caenorhabditis elegans*, HSF-1 activity was shown to be required for defense against pathogens, but the mechanism is poorly understood.

We performed bioinformatic analyses using comparative transcriptomic methods suggesting that altering HSF-1 activity may have multiple consequences on the expression of a great variety of immunity related genes in *C. elegans*. Heat shock-induced HSF-1 may enhance immunity primarily through activation of genes encoding heat shock proteins, collagens and specific immune genes. Notably, overexpression of HSF-1 that also leads to improved immunity does not activate heat shock genes and collagens. Instead, it elevates the expression of several genes involved in immunity. Interestingly, in the absence of HSF-1, genes involved in immunity are also induced. These genes mainly encode components of the intracellular pathogen response (IPR), an adaptive response to viruses and other intracellular parasites. Our studies suggest that HSF-1 does not directly inhibit this process, but that proteotoxic stress resulting from reduced HSF-1 activity may lead to IPR induction.

Currently we are investigating whether altering HSF-1 activity influences immunity against pathogen bacteria and intracellular pathogens. Our results suggest that altering HSF-1 activity may impact on *C. elegans* immunity in multiple ways and our analysis may lead to a better understanding of the role of the evolutionarily conserved HSF-1 transcription factor in immunity also in higher organisms.

Industrial-Scale Cu-TiO₂ Nanoparticle Coatings for Antimicrobial Functionalization of Recycled Feather Fillings

Mónika Vörös¹, Csaba Vágvölgyi¹, Imre Dékány², László Janovák², Csilla Veres¹

¹*Department of Biotechnology and Microbiology, Faculty of Science and Informatics, University of Szeged, H-6726 Szeged, Hungary*

²*Department of Physical Chemistry and Materials Science, University of Szeged, H-6720 Szeged, Hungary*

The reuse of cleaned feathers as filling materials offers an environmentally favorable alternative for the bedding industry, but microbial contamination developing during storage and prolonged use may limit their hygienic quality. In the present study, Cu-TiO₂ nanoparticle-based formulations were investigated as antimicrobial surface treatments for recycled feather fillings, with particular emphasis on their applicability under industrial processing conditions, resistance to mechanical and washing-related stress, and biocompatibility.

Aqueous Cu-TiO₂ suspensions containing 1.36–6.75% nanoparticles were applied to feather samples using industrial-scale wet and dry coating procedures. The antimicrobial performance of the treated materials was examined against *Cladosporium herbarum*, an allergenic filamentous fungus, and *Staphylococcus aureus* as a representative bacterial test organism. The persistence of the antimicrobial effect was assessed following abrasion, washing, and rinsing, while cytotoxicity was evaluated using HaCaT human keratinocyte cells.

Mechanical abrasion had only a limited effect on the antimicrobial properties of the coated feathers, and several formulations maintained pronounced antifungal activity after this treatment. In contrast, washing and intensive rinsing generally resulted in a reduction of antimicrobial efficacy. The most favorable antifungal performance was observed with the 2.14% Cu-TiO₂ polymer-containing formulation, which proved effective when applied by either wet or dry coating technology. Importantly, none of the industrially treated feather samples showed detectable cytotoxicity toward HaCaT cells.

These results demonstrate that Cu-TiO₂ nanoparticle coatings can be successfully integrated into industrial feather-treatment processes and may provide a feasible approach for producing recycled feather fillings with enhanced and persistent antimicrobial properties while maintaining suitable biocompatibility.

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Comparative Analysis of core Bacterial and Fungal Rhizosphere Communities in *Mentha*

Yasmine Wazzani^{1,2}, Szilvia Tavaszi-Sárosi³, Katalin Patonay⁴, Ferenc Olasz¹, Ákos Juhász^{1,*}, Katalin Posta¹

¹Agribiotechnology and Precision Breeding for Food Security National Laboratory, Institute of Genetics and Biotechnology, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

²Doctoral School of Natural Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

³Department of Medicinal and Aromatic Plants, Institute of Horticultural Science, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary

⁴Food and Wine Knowledge Centre, Eszterházy Károly Catholic University, Eger, Hungary

The rhizosphere microbiome plays an important role in plant soil interactions and consists of both conserved and variable microbial components. However, the extent to which microbial communities are conserved across *Mentha* accessions and whether these patterns differ between bacterial and fungal communities remains poorly understood. This study investigated the composition, diversity, and shared microbial fractions of rhizosphere microbiomes associated with five *Mentha* accessions cultivated under common field conditions. Five field grown accessions, *M. spicata* B1, *M. × villosa* B10, *M. longifolia* B12, *M. × piperita* B20, and *M. suaveolens* J17, were sampled at full flowering. Rhizosphere and adjacent bulk soil samples were analyzed using 16S rRNA gene and ITS amplicon sequencing, together with soil physicochemical characterization. Bacterial and fungal community composition differed significantly among accession plots, and their Bray-Curtis dissimilarities were moderately but significantly correlated. Prevalence-based core analysis revealed a marked kingdom-level contrast: at a $\geq 2/3$ within-plot prevalence threshold, 96.1% of bacterial ASVs were shared across all five accessions, whereas only 21.9% of fungal ASVs were shared. This asymmetry persisted after rarefaction and across alternative prevalence thresholds. Soil physicochemical variables were collectively associated with both communities, though no single soil parameter had an independent effect. Bacterial communities were thus characterized by an exceptionally large and abundant shared core, whereas fungal communities contained a substantially larger variable fraction, although this shared fungal component still accounted for most fungal reads, suggesting that common dominant taxa coexist with a more diverse pool of low-abundance variable taxa. These findings underscore the importance of considering both prevalence and abundance when characterizing plant-associated microbiomes, and provide evidence for distinct patterns of bacterial and fungal community organization under common field conditions.

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