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11th International Doctoral Students' Conference in Life Sciences

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14-15 MAY 2026
LODZ | POLAND

BOOK OF ABSTRACTS

BIOOPEN WE ARE UNIC! | FACULTY OF BIOLOGY AND ENVIRONMENTAL PROTECTION University of Lodz UNIC

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Faculty of Biology and Environmental Protection

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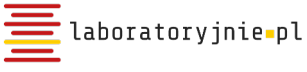









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PROF. PIOTR MINIAS

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Prof. Piotr Minias is a lecturer and researcher, and holds a position as Head of Department of Biodiversity Studies, Teacher Training and Bioeducation at the University of Lodz. Since 2010, he has been awarded multiple competitive research grants funded at the national level. His research work examines how environmental factors and evolutionary pressures shape key biological traits with particular emphasis on genetic polymorphism and evolution of genes encoding immune molecules and receptors in wild bird populations. A central focus of his research also involves ecological and evolutionary responses of birds to ongoing environmental change. His research contributions are widely cited and frequently involve international collaboration. He is the author or co-author of more than 180 publications, with a Hirsch index of 21.

Genetic diversity at the extremes: what the most polymorphic vertebrate genes reveal about host-parasite interactions

Host-parasite interactions have long been of central interest to ecologists and evolutionary biologists. Research on antigen-recognition genes, such as the Major Histocompatibility Complex (MHC), offers unprecedented insights into eco-evolutionary dynamics between hosts and their parasites. The MHC region contains the most polymorphic genes in vertebrate genomes, and balancing selection effectively maintains extraordinary diversity of MHC genes in many natural vertebrate populations. At the same time, the accelerating accumulation of high-quality genomic resources opens novel avenues for a broad-scale comparative investigation of evolutionary mechanisms underlying this hyper-polymorphism. Here, I will present our research on the ecological and evolutionary drivers of MHC diversity in birds. In particular, I will explore whether and how MHC diversity is shaped by species life history traits and how it responds to novel anthropogenic selective pressures. I will also discuss how MHC diversity relates to fitness, and whether it may have conservation implications for wild birds.

PROF. BEGOÑA GARCÍA-ZAPIRAIN

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Begoña García-Zapirain was born in San Sebastian (Spain) in 1970. She graduated in Communications Engineering, specializing in Telematics at the Basque Country University, Bilbao (Spain) in 1994. In 2004, she defended her doctoral thesis in biomedical signal processing research area. After four years of working for ZIV Company, in 1997, Prof. García-Zapirain joined the University of Deusto as a lecturer in signal theory and electronics at the Engineering School. She was the head of the Telecommunication Department at the University of Deusto from 2002-2008 and head of DeustoTech-LIFE Department from 2008-2018 and nowadays is leading the Biomedical Engineering program. In 2001 she created eVIDA research group (evida.deusto.es). She has participated in more than 120 research projects on international, national and regional levels, published more than 150 papers in international scientific ISI indexed journals, and presented more than 160 papers in international and national scientific conferences. She has been supervising 20 defended theses and has ongoing 5 PhD students. She has strong collaborations with research labs in USA, UK, France, Ireland, Belgium, Finland and Poland among others. She has 6 research awards at national level.

Health 4.0: Digital Transformation of Healthcare Systems and Real-World Use Cases

Health 4.0 represents the evolution of healthcare systems through the integration of advanced digital technologies such as Artificial Intelligence, Internet of Medical Things (IoMT), Artificial Intelligence, augmented and virtual reality, cloud computing, and cyber-physical systems. This lecture will explore how these technologies are reshaping prevention, diagnosis, treatment, and patient monitoring. Special emphasis will be placed on real-world use cases demonstrating the implementation of intelligent, patient-centered, and data-driven healthcare solutions, highlighting both technological challenges and clinical impact.

ASSOC. PROF. YANN SEIMBILLE

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Consistent, yet flexible and a keen observer with a Doctorate in Radiopharmaceutical Sciences. Experience carries technical and practical involvement in a broad range of translational research projects on radiopharmaceuticals. Gained exceptional skills and knowledge in chemistry and radiopharmaceutical sciences through rigorous training at the University of Paris XI, University of Sherbrooke and the University of California, Los Angeles (UCLA). Harnessed in-depth knowledge of organic chemistry, radiochemistry, analytical chemistry and pharmacology. Proven track record of implementing innovative solutions in a challenging and dynamic atmosphere.



Development and preclinical evaluation of novel theranostics for imaging and treatment of cancers

Radiopharmaceuticals play a pivotal role in modern medicine by enabling both non-invasive imaging and targeted therapy. Diagnostic agents, used with techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT), allow visualization of pathophysiological processes, while therapeutic radiopharmaceuticals deliver cytotoxic radiation selectively to malignant tissues. Our research focuses on the development and clinical translation of theranostics that integrate diagnostic and therapeutic functionalities. We design radiopharmaceuticals by optimizing key chemical and biological parameters, including binding affinity, specificity, stability, and pharmacokinetics, alongside radionuclide properties. Peptides and small molecules are employed as targeting vectors due to their favorable pharmacokinetics and tissue penetration. We synthesized novel compounds targeting clinically relevant biomarkers such as SSTR2, GRPR, PSMA, and FAP, with multimerization strategies enhancing tumor uptake and retention (e.g., eFAP-51). Lead candidates are advancing through (pre)clinical evaluation. In targeted alpha therapy, we investigated alpha-emitting radionuclides (e.g., lead-212, actinium-225) to improve peptide receptor radionuclide therapy. Notably, [212Pb]Pb-eSOMA-01 demonstrated higher tumor uptake and reduced renal dose compared to current standards, highlighting its therapeutic potential for neuroendocrine tumors. We also developed theranostic small-molecule drug conjugates (T-SMDCs), combining targeting ligands with cytotoxic payloads such as DM1 and radiolabeling capability, enabling integrated imaging and therapy. Additionally, we explored fluorescence-guided surgery using novel probes (e.g., eFAP-24) for real-time tumor delineation. Together, these approaches aim to advance precision oncology through improved diagnosis, as well as safer and more effective treatments.

ASSIST. PROF. GABRIEL ARISMENDI MORILLO

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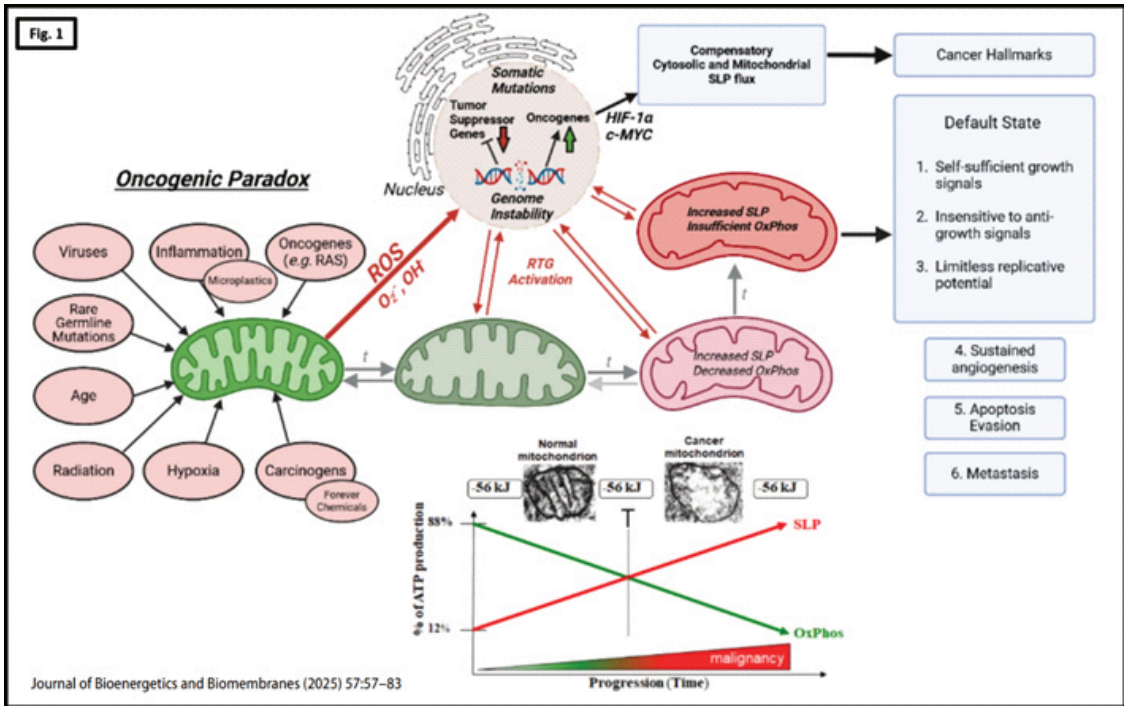
Prof. Gabriel Arismendi-Morillo, MD is a lecturer and researcher at Biological Research Institute, Faculty of Medicine at the University of Zulia, Marcaibo City, Venezuela and Department of Medicine, Faculty of Medicine at the University of Duesto, Bilbao, Spain. He is a Chief of Anatomic Pathology Department and Pathologist Consultant at Hospital General del Sur "Dr. Pedro Iturbe" and Coordinator at Electron Microscopy Laboratory and Gastrointestinal Research Laboratory at Biological Research Institute. His research focuses on the role of mitochondrial structure and dysfunction in human cancer, with particular emphasis on brain tumors such as glioblastoma. He has contributed to studies investigating mitochondrial abnormalities, including mitochondria-associated membranes (MAMs), and their role in tumor metabolism, apoptosis and cellular signaling. His work further integrates the histopathological analysis of tumors, cancer metabolism, and the development of novel therapeutic approaches. Prof. Arismendi-Morillo is the author or co-author of more than 50 publications and is an active reviewer for top-tier journals. With 120 contributions to national and international scientific conferences and congresses, he has also been recognized with 24 national and international awards and distinctions.

Mitochondrial Pathobiology is the α and ω of cancer and an open door for non-toxic therapies?

Mitochondrial pathobiology represents the basis of metabolic and bioenergetic hallmarks in human cancer. The metabolic reprogramming in cancer cells, is linked to chronic mitochondrial cristae lesion (named α). Consequently, most cancer cells with mitochondrial cristae defects would be incapable of producing adequate amounts of energy through oxidative phosphorylation. ATP production through increased glucose-driven cytosolic and glutamine-driven mitochondrial substrate-level phosphorylation thus becomes necessary to compensate for OxPhos insufficiency (Figure 1). The metabolic targeting (i.e. simultaneous restriction of the glycolysis and glutaminolysis pathways) starve the tumor cells and induces a progressive and continuous process of tumor cell death (named ω), with the subsequent pathological and clinical responses derived from the interplay between the functional state of mitochondria and their environmental signals (Figure 2). It is imperative the study the molecular and ultrastructural pathology of mitochondria, mitochondrial cristae, mitochondrial contact site and cristae organizing system (MICOS), mitochondria endoplasmic reticulum contacts (MERCS), intercellular mitochondrial transfer, as well as, cell death mechanisms (apoptotic cell death, autophagic-dependent cell death, autoptic cell death, ferroptosis, pyroptosis) in response to the therapeutic approaches. Finally, it will be necessary to expand the clinical testing of diet-drug combinations, such as, ketogenics diets and fasting alongside glycolysis and glutaminolysis inhibitors, to establish, the optimal administration protocols that leverage the structural and functional abnormalities described in tumor mitochondria (Figure 3).



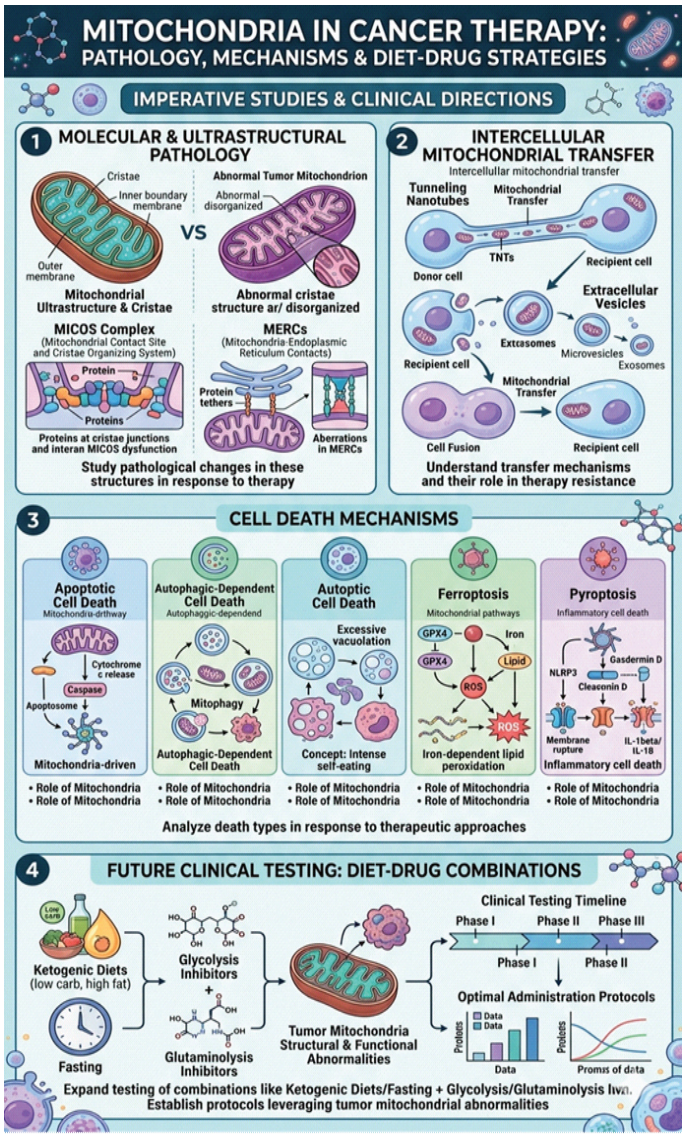
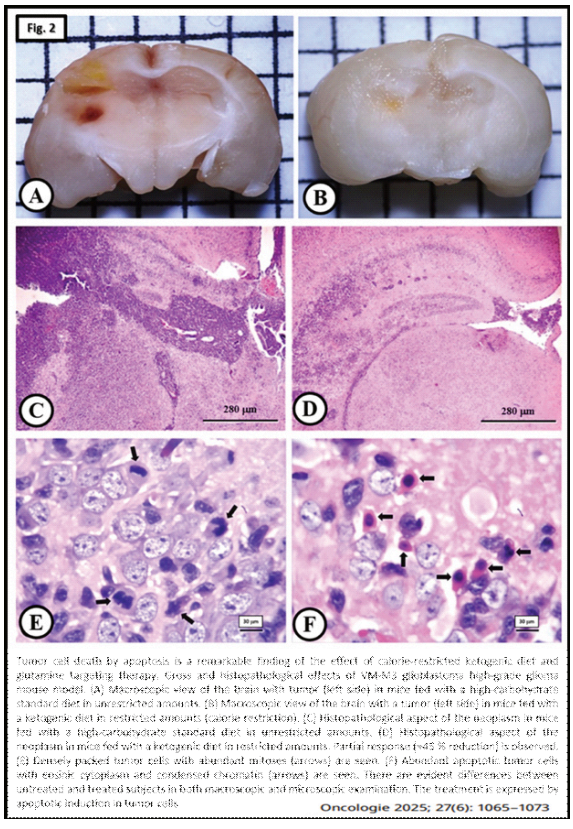


Figure 3. This figure was created with Google Gemini (https://gemini.google.com/)



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Sandra Hudina graduated in Biology (Ecology) from the University of Zagreb in 2002, obtained her MSc from the University of Oxford (UK) in 2006, and earned her PhD in 2012 at the University of Zagreb. She is currently Associate Professor at the Department of Zoology, Faculty of Science, University of Zagreb, where she has been employed since 2007. She completed research stays at Wageningen University (Netherlands) and the University of St. Thomas (USA).

Her research focuses on invasion ecology, freshwater biology, and host-associated microbial communities. She has co-authored more than 40 peer-reviewed publications, serving as first or corresponding author on over 20 publications. She has been a leader of several research projects and participated in a number of national and international projects.

Sandra Hudina teaches at undergraduate and graduate levels and has supervised numerous MSc and undergraduate theses, as well as one PhD dissertation. She has received the Faculty Council Award for scientific excellence (2012) and the Student Council Award for Best Assistant (2016).

What Makes an Invader Successful? Insights from the Signal Crayfish

Invasive non-native species threaten biodiversity, human well-being, and the global economy. Although only a tiny proportion of all introduced species worldwide manage to establish, spread, and generate substantial ecological impacts, those that do can have far-reaching environmental, economic, and public health consequences. Understanding what drives invasion success therefore remains one of the key questions in invasion biology.

Freshwater biodiversity has experienced dramatic declines in recent decades due to multiple interacting anthropogenic pressures, including the introduction and spread of non-native species. Although crayfish play important roles in maintaining ecosystem structure and function in their native ranges, translocations for aquaculture and the ornamental trade have led to their widespread introductions across Europe. Many of these species have rapidly expanded and became among the most prominent invaders of European freshwater ecosystems.

In this presentation, I will summarize findings from over a decade of research on invasion success determinants in one of Europe's most widespread crayfish invaders, the signal crayfish (Pacifastacus leniusculus). Our studies are based on comparisons of populations along invasion gradients in Croatia, contrasting long-established, high-density core populations with recently established populations at the invasion front. This approach enabled us to assess differences in population structure, individual condition, behavior, immune response, and microbiome composition during range expansion. The results are discussed in relation to population dynamics, trait-based advantages in newly colonized environments, and the contribution of individuals' traits to the competitive performance of invasive species.

ABSTRACTS:

MICROBIOLOGY, IMMUNOLOGY, AND BIOTECHNOLOGY

IFN-γ induces genes are involved in the pyroptotic death of human cells upon bacterial infection

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Interferons (IFNs) are potent cytokines which accelerate innate response to infection through enhancing expression of interferon stimulated genes (ISGs). To this group belongs gunaylate binding proteins (GBPs), caspase 4 (CASP4) or gasdermin-D (GSDMD) that facilitate recognition of the bacterial invaders in the cytosol and execute host defense functions. GBP1 exhibits a remarkable ability to: (i) bind and cover intracellular pathogen surface, (ii) cause disruption of lipopolysaccharide (LPS) structure, and (iii) recruit other family members (GBP2-4) to the bacteria leading to CASP4 recruitment and activation for execution of pyroptotic cell death. However, considering the serious consequences, which death brings for infected cell, we propose that other IFN-γ-regulated proteins are likely to be involved in the process as well.

Main purpose of this study was to find yet not determined, but necessary factors that take part in the pyroptotic cell death pathway activated by the LPS of intracellular pathogens. To fulfill the goal a custom-made library of silencing RNA (siRNA) targeting IFN-stimulated genes was applied and cells were infected with *Shigella flexneri*. This approach allowed us to determine host factors, which can facilitate signaling pathway leading to pyroptosis. We confirmed their significance for the cell death by construction of siRNA resistant gene variants. Additionally, we endeavor to determine their role during infection by different molecular and cell biology methods.

Obtained results allow us to understand better the interactions between host and pathogen, what may be pivotal to create new vaccines or drugs, especially in the post-antibiotic era.

Isolation and characterization of plant growth-promoting bacteria with bisphenol-degrading potential

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Extensive usage of bisphenols F and S (BP_F, BP_S) carries several environmental and human risks. These compounds, widely employed in the production of plastics, resins, and thermal paper, have been identified as endocrine disruptors that interfere with hormonal balance and may lead to reproductive disorders in humans. Additionally, their high persistence in the environment contributes to widespread contamination of water, soil, and food. Therefore, research on environmentally friendly methods of removing bisphenols from polluted soil and water should be designed and tested. One of those methods is phytoremediation assisted by bacteria able to degrade BP_{FS} and promote plant growth. The aim of the presented studies was to isolate bacterial strains suitable for supporting the phytoremediation of environments contaminated with BP_F and BP_S.

In the current study, 39 bacterial strains were isolated from tissues (leaves and roots) and the rhizosphere of *Solidago virgaurea* L. and *Elymus repens*, growing near the waste dump in Tarnowskie Góry and the Institute of Heavy Organic Synthesis „Blachownia" in Kędzierzyn Koźle, Poland. Both locations are associated with intensive industrial and waste-related practices, which may lead to the presence of organic pollutants, such as bisphenols, in the surrounding environment. All isolated bacteria were analyzed for their ability to degrade bisphenol F or S, and to promote plant growth (PGP). Bacteria were isolated on BSM medium supplemented with BP_F/BP_S as the only carbon and energy source. In the next step, strains were tested for their abilities to produce siderophores, cellulolytic enzymes, ACC deaminase, indole-3-acetic acid (IAA), biosurfactants, and to release phosphorus from insoluble compounds. Additionally, strains were tested for biofilm formation and motility. Finally, high-performance liquid chromatography (HPLC) was used to determine the efficiency of BP_F/BP_S degradation in liquid BSM cultures with an initial concentration of 10 mg l^{-1}.

Among the 39 bacterial isolates, 46% were capable of producing IAA, and 10% produced siderophores. Notably, ACC deaminase activity was observed in 87% of the isolates. Additionally, 26% of the isolates demonstrated the ability to form biofilms, potentially favoring their colonization of plant roots and survival under harsh environmental conditions. Despite extensive screening studies, only strain E12 showed effective bisphenol S degradation. HPLC analysis showed that this strain degraded 36% of the initial BP_S.

The obtained results indicate that effective bisphenol-degrading bacteria are relatively rare, even in highly contaminated environments; however, plant-associated bacteria from industrially impacted sites may possess both plant growth-promoting traits and the ability to partially degrade bisphenols, highlighting their potential application in bioaugmentation-assisted phytoremediation.

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Phenotype and genotype characteristics of *Listeria monocytogenes* strains isolated from wastewater treatment plants

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Listeria monocytogenes is a Gram-positive rod-shaped bacterium that is widely distributed in the environment and resistant to many stress factors. Sources of *L. monocytogenes* include meat and milk products, fish and fish products, vegetables and fruits. The bacterium is the etiological agent of listeriosis, a zoonotic disease which has the ability to cause invasive infections and miscarriages in humans. Evaluation of the virulence of *L. monocytogenes* isolates is important for assessing the risk of infections resulting from contamination of crops and drinking water sources.

The aim of the study was to characterize the phenotype and genotype of L. monocytogenes isolated from wastewater treatment plants. The research material consisted of 57 strains of *L. monocytogenes* isolated from wastewater treatment plants and a hospital collectors in the Kuyavian-Pomeranian Voivodeship. Isolates were collected between January and May 2025. Species identification was performed using MALDI TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry). The species affiliation of the isolates was confirmed by multiplex polymerase chain reaction (PCR). The isolates were tested for antibiotic susceptibility to penicillin, ampicillin, meropenem, erythromycin, and cotrimoxazole using the disk-diffusion method. Affiliation of isolates to serogroups (1/2a-3a, 1/2b-3b, 1/2c-3c, 4b-4a-4d) and presence of selected virulence genes (*fbpA, plcA, hlyA, plcB, inlB, actA, iap, inlA, mpl* and *prfA*) were examined using multiplex PCR method.

The dominant profiles of antibiotics susceptibility were profile I (n=22; 38.60%) comprising isolates susceptible to all tested antibiotics and profile X (n=15; 26.32%) – isolates resistant to penicillin, ampicillin, and meropenem, retaining sensitivity to erythromycin and cotrimoxazole. The presence of one isolate resistant to all tested antibiotics was confirmed. The highest number of strains belonged to serogroup 1/2a-3a (n= 29; 51.0%) and 4b-4a-4d (n= 19; 33.0%). Among *L. monocytogenes* isolates, a variable frequency of genes encoding virulence factors was demonstrated. The presence of the *plcA, hlyA, actA, iap,* and *prfA* genes was detected in all fifty-seven strains. Isolates carrying all of the virulence genes tested were identified.

Results confirmed that wastewater treatment plants are a source of *L. monocytogenes* isolates with diverse antibiotic susceptibility profiles and presence of virulence genes. This highlights the need to monitor the environment of wastewater treatment plants as a potential source of virulent strains of *L. monocytogenes*.

Prevalence of antibiotic-resistant *Enterococcus* spp. and *Staphylococcus aureus* strains in wastewater treatment plant environments

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Antimicrobial resistance (AMR) is a major global health threat, with wastewater recognized as a potential reservoir and dissemination route for resistance determinants. The presence of diverse microorganisms and residual antibiotics in wastewater may promote the selection and horizontal transfer of resistance genes.

This study aimed to assess the prevalence of antibiotic-resistant *Enterococcus* spp. and *Staphylococcus aureus* isolated from hospital and municipal wastewater, and to detect selected resistance genes among resistant strains.

Material for the study consisted of 131 wastewater samples. Isolation of *Enterococcus* spp. and *Staphylococcus aureus* strains was performed using selective and chromogenic media. Species identification was conducted by MALDI-TOF MS. Minimum inhibitory concentrations (MICs) for glycopeptides were determined for *E. faecium*, *E. faecalis*, and *S. aureus*, while susceptibility to other antibiotics was assessed using the disk diffusion method. Antimicrobial susceptibility was interpreted using EUCAST v 15.0 guidelines. Antibiotic-resistance genes (*vanA*, *vanB*, and *mecA*) were detected by PCR.

Out of collected samples, 80 (69.6%) *E. faecium* strains, 21 (18.3%) *E. faecalis* strains and 14 (12.2%) *S. aureus* strains were isolated. Most of the microorganisms (n=33; 28.7%) were isolated from raw sewage. Among the tested strains, 100 (87.0%) were resistant to at least one antibiotic. Vancomycin-resistant *E. faecium* (VRE) accounted for 95.0% (n=76) of *E. faecium* isolates, while methicillin-resistant *S. aureus* (MRSA) represented 28.6% (n=4) of *S. aureus* strains. Enterococci most frequently showed resistance to fluoroquinolones (79.2%), while among *S. aureus* isolates, the largest number of strains were resistant to ceftaroline (n=11; 78.6%). Multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes were identified in 50 (43.5%) and 32 (27.8%) isolates, respectively. The *vanA* gene was predominant among VRE strains (60%), and all MRSA isolates carried the *mecA* gene.

These findings confirm the high prevalence of antimicrobial-resistant bacteria in wastewater and highlight the need for continuous environmental surveillance to limit the spread of resistance genes.

Acknowledgments

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Antifungal activity of Volatile Organic Compounds (VOCs) of *Trichoderma atroviride* in the presence of heavy metal ions

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The last decade has seen an increase in interest in VOCs in agricultural research, reflecting their potential use in biological plant protection. VOCs constitute a significant portion of the total metabolites emitted by microscopic fungi. Among these fungi, representatives of the *Trichoderma* genus stand out, with isolates demonstrating high efficacy in controlling phytopathogenic fungi in soil, including *Fusarium oxysporum*. A better understanding of the VOCs action produced by *Trichoderma* spp. may contribute to the effective use of these fungi in practice.

The aim of this study was to evaluate the effect of Zn^{2+} and Cu^{2+} on the production of VOCs emitted by *Trichoderma atroviride* and to determine whether heavy metal ions influence the antagonistic activity of *T. atroviride* against *F. oxysporum* infecting wheat seedlings. The experiment used *Triticum aestivum* L. seeds placed in Petri dishes. *T. aestivum* L. seeds were treated with a suspension of fungal spores of the *T. atriviride* KKP 3765 and *F. oxysporum* DSM 1094, as well as Zn^{2+} and Cu^{2+} at the appropriate concentrations. Activated carbon was added to the plates to enable efficient VOCs extraction. The plates were incubated in a laboratory phytotron for 7 days. Qualitative analysis of VOCs emitted by *Trichoderma* fungi was performed using the GC-MS method and quantitative analysis performed using the internal standard.

Analysis showed that *T. atroviride* against with *F. oxysporum* produced a characteristic profile of volatile organic compounds. A total of 27 different volatile substances were detected, including 2-acetylcyclopentan-1-one, 2,4-dimethylcyclopentan-1-one, 1-cyclopentylethanone and other metabolites. Quantitative analysis of VOCs was also carried out. Interestingly, in the presence of Zn^{2+} and Cu^{2+}, VOCs production by *T. atroviride* against *F. oxysporum* was significantly inhibited. In the presence of Zn^{2+}, only two volatile substances were detected, and in the presence of Cu^{2+}, five volatile substances were detected. In *Trichoderma-Fusarium* systems supplemented with Zn^{2+} or Cu^{2+}, significant deterioration of leaf and root growth was observed compared to the system containing only *Trichoderma-Fusarium*. In the presence of Zn^{2+}, growth inhibition was approximately 52% for leaves and 48% for roots, while in the presence of Cu^{2+}, inhibition was approximately 42% for leaves and 35% for roots. These results suggest that inhibition of VOCs produced by *T. atroviride* in the presence of heavy metals negatively impacts the effectiveness of its interaction with the plant.

The study showed that the presence of Zn^{2+} and Cu^{2+} significantly reduced the ability of *T. atroviride* to release VOCs against *F. oxysporum*, resulting in impaired plant growth. VOCs production plays a significant role in indirectly affecting leaf and root growth of *T. aestivum* L., and their inhibition under metal stress conditions reduces the effectiveness of this interaction.

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Microbial competition as a factor determining the efficacy of bacterial-assisted phytoremediation

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The aim of this study was to evaluate the impact of soil microbial competition on the phytoremediation efficiency of an aged petroleum hydrocarbon-contaminated soil. This soil was collected from an industrial area near a 100-year-old oil refinery located in Czechowice-Dziedzice (Upper Silesia, southern Poland). The initial total petroleum hydrocarbon (TPH) concentration was 22,590 ± 3,378 mg kg^{-1} dry weight (d.w.) of soil, measured independently for each pot before and after the bioremediation study following the ISO 16703:2011 protocol. Sterilized (S) and non-sterilized (NS) soils were planted with *Zea mays* and bioaugmented with *Pseudomonas allii* CZK2 and *Ralstonia picketii* CZR6 applied as single inoculants.

The phytoremediation experiment was conducted under controlled laboratory conditions and included the following treatments: (1) planted soil inoculated with live CZK2 strain, (2) planted soil inoculated with thermally inactivated CZK2 strain (lack of survival), (3) planted soil inoculated with live CZR6, (4) planted soil inoculated with thermally inactivated CZR6 (lack of survival), and (5) planted soil treated with water (natural attenuation, NA).

At the end of the experiment, the following parameters were assessed: (i) TPH removal efficiency, (ii) plant biomass production, (iii) total bacterial abundance, and (iv) the proportion of bacteria with the potential to degrade aliphatic hydrocarbons.

Compared with the natural attenuation experimental group, significantly higher TPH removal efficiency was observed in S soil inoculated with live CZK2 cells, S soil treated with thermally inactivated CZK2 cells, and NS soil inoculated with live CZR6 cells. The mean reductions in TPH concentration across these treatments were 63%, 44%, and 40% of the initial hydrocarbon concentration, respectively. In NS soil, biostimulation with thermally inactivated CZK2 cells promoted root growth, increasing its biomass by 33.5% compared to the NA, whereas bioaugmentation with live CZR6 cells significantly enhanced shoot biomass and length, increasing these parameters by 19.2% and 11.4%, respectively, compared to the NA variant. In S soil, biostimulation with dead CZK2 biomass had the strongest positive effect on plant performance, significantly increasing both root and shoot biomass by 34.3% and 39.2%, respectively. None of the applied treatments significantly affected 16S rRNA gene copy numbers in NS soil compared with the NA. In contrast, biostimulation of S soil with dead CZK2 biomass significantly increased 16S rRNA gene abundance, reaching 10.53 $\log_{10}$ gene copies g^{-1} d.w. of soil, compared to the NA (9,64 $\log_{10}$ gene copies g^{-1} d.w. of soil). Neither bioaugmentation nor biostimulation with the tested strains significantly altered the abundance of genes involved in hydrocarbon degradation. The copy number of the cytochrome P450-type alkane hydroxylase gene (CYP153) remained comparable to the NA in both NS and S soils and within a range of 9.72-10.12 and 9.28-9.98 $\log_{10}$ gene copies g^{-1} d.w. of soil, respectively. **Conclusions**: (i) competition between introduced strains and autochthonous soil communities is a key factor influencing bioaugmentation efficacy, (ii) higher remediation efficiency observed in sterilized soil indicates that competition with soil autochthonous microorganisms limited the functional activity of inoculants.

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Microbial biodiversity in honey as a source of potential probiotics

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Honey is a natural product known for its antimicrobial activity; however, it may also serve as a reservoir of beneficial microorganisms. The aim of this study was to isolate bacteria from honey samples and evaluate their probiotic potential based on selected biochemical and physiological criteria.

Eighteen bacterial strains were isolated and subjected to assessment of hemolytic activity, tolerance to bile salts, survival under acidic conditions, catalase activity, and carbohydrate metabolism profiles. The results demonstrated considerable variability among isolates. Several strains exhibited resistance to bile salts and maintained viability at low pH, indicating their ability to survive gastrointestinal-like conditions. Non-hemolytic behavior and diverse carbohydrate metabolism patterns further supported the probiotic potential of selected isolates.

These findings suggest that honey may represent a source of bacterial strains with promising probiotic characteristics, warranting further molecular identification and in vivo evaluation.

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The effect of pyrethroids on the viability of *Samsoniella alboaurantium*

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Introduction. Pyrethroids are a group of synthetic insecticides. Their common use leads to accumulation in various ecosystems. An alternative to chemical control is the use of entomopathogenic fungi, such as *Samsoniella* species, which serve as biopesticides. However, those fungi are also exposed to the residues of pesticides in the environment. Therefore, understanding the impact of pyrethroids on the viability of entomopathogenic fungi is crucial.

Objectives. This study investigates the effect of pyrethroids – λ-cyhalothrin and α-cypermethrin on the viability of *Samsoniella alboaurantium* 240.32.

Materials and Methods. To each well of a 96-well plate, 100 µl of spore suspension was added to 100 µl of a mixture of Lobos medium with 1% glucose and the pyrethroids at following concentrations (mg/L): 0.16, 0.31, 0.63, 1.25, 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0. The abiotic control consisted of 200 µl of medium, and the biotic control consisted of 100 µl of medium and 100 µl of spore suspension. The plate was incubated for 48 hours at 28°C. Viability was determined using the FDA (Fluorescein Diacetate Assay), and fluorescence intensity was measured at excitation/emission wavelengths of 485/520 nm.

Results. Both pyrethroids demonstrated a dose-dependent effect on the viability of *Samsoniella alboaurantium* strain 240.32. λ-cyhalothrin exhibited higher toxicity, with viability dropping below 50% at concentrations ranging from 20 to 80 mg/L. In contrast, α-cypermethrin reached this toxicity threshold only at 40 and 80 mg/L, maintaining a high viability of 79% at the 20 mg/L concentration. The viability of the fungus remained relatively high at α-cyhalothrin concentrations up to 10 mg/L.

Conclusions. A downward trend was observed in *Samsoniella alboaurantium* 240.32 strain viability with increasing concentrations of pyrethroids. λ-cyhalothrin demonstrates higher toxicity than α-cypermethrin; the critical threshold where viability drops below 50% is reached at smaller concentration for λ-cyhalothrin (20 mg/L) compared to α-cypermethrin (40 mg/L). However, the strain was not completely inhibited within the tested concentration range, suggesting partial tolerance to the analyzed pyrethroids.

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Assessment of the stability of selected formulations based on essential oils in terms of their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*

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The increasing prevalence of antimicrobial resistance among environmental and opportunistic bacteria represents a major global public health challenge. The widespread use and misuse of antibiotics have accelerated the emergence of resistant strains not only in clinical settings but also in environmental reservoirs, which contribute to the dissemination of resistance genes. Among the most significant opportunistic pathogens are *Escherichia coli* and *Staphylococcus aureus*, microorganisms commonly associated with both community-acquired and healthcare-related infections. The increasing prevalence of methicillin-resistant *S. aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing *E. coli* highlights the urgent need to explore alternative antimicrobial strategies. In recent years, naturally derived agents, particularly plant extracts, have gained increasing popularity as potential alternatives to conventional chemical disinfectants due to their broad-spectrum antimicrobial properties and perceived environmental safety. However, despite the growing interest in such natural products, the issue of their long-term stability and persistence of antimicrobial activity during storage remains insufficiently addressed in the scientific literature.

The aim of this study was to compare the antibacterial activity and storage stability of selected essential oil-based formulations against reference strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). Three formulations differing in the composition of essential oils were evaluated. The tested compositions included cinnamon bark, bergamot, clove, thyme, coriander, eucalyptus, lemon, oregano, and tea tree essential oils. Additionally, all tested formulations contained Tween as a solubilizing agent, ethanol, and water.

Antibacterial activity was assessed using the agar well diffusion method on Mueller-Hinton agar plates. Fresh bacterial suspensions of *E. coli* and *S. aureus* were prepared and used to inoculate agar surfaces. Wells were filled with 20 µL of each tested formulation or negative control. Antibiotic discs containing tetracycline (TE30) and ciprofloxacin (CIP5) served as positive controls. Plates were incubated at 37 °C for 24 hours, after which inhibition zones were measured and documented. Stability of antibacterial activity was evaluated by comparing inhibition zones over the long storage period (up to one month).

In conclusion, all investigated essential oil biopreparations demonstrated antibacterial activity against *E. coli* and *S. aureus*, although their effectiveness varied among the tested formulations. In selected formulations, antibacterial activity changed during storage depending on the specific formulation and, in some cases, the bacterial strain tested. Stability studies are therefore indispensable to accurately define shelf life, ensure sustained antimicrobial performance, and support the safe and effective application of natural biopreparations in practical settings.

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Tracking aggregation of Sup35 protein during osmotic and ER stress

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Sup35 protein forms amyloid aggregates in yeast cells, called [*PSI*] prion, and affects cell stress response and phenotype [1]. Using flow cytometry and aggregation-sensing genetic construct [2], we track aggregation levels of Sup35 during osmotic and ER stress and the effect of guanidine hydrochloride (GuHCl).

Hsp104 inhibition by guanidine hydrochloride is known to cause Sup35 protein aggregates to grow in size, and formation of these megaDalton-sized aggregates mediates prion curing by disallowing prion fibrils to be inherited by daughter cells. While cellular stress is expected to induce chaperone expression which could fragment [*PSI*] aggregates and propagate prion to daughter cells. In this work, we use yTrap system [2] which works by fusing Sup35 to synthetic transcription factor, the aggregation of this construct "turns on" GFP expression in concentration dependent manner. 74-D694 strain yeast cells were treated with 2 M sorbitol for osmotic stress or 1 mM DTT for ER stress in rich growth media YPD, recovered, fixed and measured on BD FACS A1 flow cytometer. Data was normalized to GFP fluorescence in [*PSI*] prion-lacking cells.

[*PSI*] strong cells treated with osmotic stress and GuHCl alone showed similar GFP+ populations (1.8% vs. 2.7%), but combined treatment increased GFP+ population to 17% of gated cells. Under ER stress, 0.95% of gated cells were GFP positive, while combined GuHCl and DTT treatment increased GFP+ cell population to 10.7% of all gated cells.

In [*PSI*] weak cells, 10.5% GuHCl-treated cells were GFP positive, while under osmotic stress 1.95% of cells were GFP+. Under combined GuHCl treatment and osmotic stress, 27.7% of gated cells were GFP positive. Under ER stress, 3.23% of cells were GFP+, while under combined GuHCl and DTT treatment, 18.9% of gated cells were GFP positive.

Stress increase Sup35 aggregation, as sensed by yTrap system, to a lesser degree than GuHCl treatment. However, combined GuHCl and stress increase aggregation (GFP+ cell population) to a larger degree than either treatment alone, with osmotic stress having more pronounced effect on aggregation than ER stress. [PSI] prion variant also had effect on aggregation, with weak variant showing more pronounced aggregation than strong variant. The effect of increased aggregation on [PSI] prion curing needs to be confirmed by further testing [PSI] phenotypic assays and biochemical assays as some of the sensed aggregation could be due to phase separation rather than protein aggregation.

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Aureobasidium pullulans as a component of biopreparations for the protection of cereal seeds against *Fusarium spp.*

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Filamentous fungi of the genus *Fusarium* pose a significant threat to the quality and safety of cereal grains, leading to yield losses and contamination of raw materials with mycotoxins. The continuous development and modernization of agriculture result, among other factors, from increasing consumer demand for stable supplies of high-quality, healthy food free from chemical plant protection residues. Excessive use of pesticides contributes to soil degradation and generates serious environmental problems. Consequently, there is an intensive search for natural preventive methods that ensure effective pathogen control and constitute a viable alternative to conventional chemical substances. One promising approach is the use of microorganisms for seed treatment to protect cereal grains against pathogens.

The aim of this study was to evaluate the antifungal potential of selected *Aureobasidium pullulans* strains against molds of the genus *Fusarium*. The conducted experiments enabled a detailed analysis of the inhibitory effects of *A. pullulans* strains under in vitro conditions, both as single isolates and in combinations with selected bacterial species.

The obtained results confirm the high efficacy of *A. pullulans* strains in limiting the development of *Fusarium* spp. pathogens. The most significant effect was a strong reduction in the growth of colonies of potential phytopathogens—up to 73% compared to the control—indicating a pronounced biocontrol potential. The observed compatibility of *A. pullulans* with the tested bacteria, without negative interactions, enables their application in biological preparations based on fungus–bacteria combinations. The use of such microbial mixtures demonstrated a synergistic effect in inhibiting pathogens. Complementary analyses included an assessment of carbon metabolism by *A. pullulans* using the BIOLOG Odin system, which allowed identification of preferred carbon substrates and revealed differences in the range and intensity of growth.

The conducted research demonstrates that biopreparations based on *A. pullulans* strains may constitute an effective and environmentally friendly alternative to chemical plant protection products. Their application allows limitation of the development of dangerous *Fusarium* spp. phytopathogens, which is particularly important in the context of sustainable agriculture. The results provide a solid basis for further research on the practical implementation of these solutions, as well as for optimization of production and application parameters of biopreparations in modern cereal protection.

The effect of metribuzin on the growth *of Akanthomyces muscarius* strains

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Metribuzin (4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazine-5(4H)-one) belongs to a group of agents commonly used in plant protection against pests, i.e., triazine herbicides. It is mainly used to control broadleaf weeds and grasses. It works by inhibiting electron transport in the process of photosynthesis. Due to its widespread use, persistence in the environment, and low degradation rate, residues of this compound can be foundin soil. This herbicide has been proven to have a toxic effect on organisms and the environment, which highlights the need to implement alternative crop protection measures, such as the use of entomopathogenic fungi. The main habitat of entomopathogenic fungi, which includes fungi of the genus *Akanthomyces*, is the soil environment, and thus they are exposed to pesticides commonly used in plant protection, including metribuzin. It is therefore essential to understand the impact of synthetic insecticides on the viability of fungi used in biological pest control.

The aim of the study was to examine the effect of different concentrations of metribuzin on the viability of the strains *Akanthomyces muscarius* CBS 113458 and *Akanthomyces muscarius* CBS 318.70.

The study showed that *Akanthomyces muscarius* CBS 113458 had a higher tolerance to the tested agent than *Akanthomyces muscarius* CBS 318.70. A decrease in viability below 50% was observed at a concentration of 5 mg/L of metribuzin. In contrast, the *Akanthomyces muscarius* CBS 318.70 strain showed a decrease below 50% at a concentration of 2.5 mg/L of metribuzin. The lower concentrations of the tested agent caused a minor decrease in viability in both *Akanthomyces muscarius* CBS 113458 and *Akanthomyces muscarius* CBS 318.70.

Both studied strains of entomopathogenic fungi were resistant to metribuzin concentrations higher than those found in soil, which are approximately 0.15–1.8 mg/L, indicating that they can be used in areas contaminated with metribuzin.

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Prion-variant–dependent transcriptional responses to GuHCl during heat and oxidative stress in *S. cerevisiae*

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Yeast prions are protein-based epigenetic elements that generate heritable phenotypic diversity and can intersect with cellular stress responses. Environmental stress increases the frequency of prion appearance in *Saccharomyces cerevisiae* [1]. Guanidine hydrochloride (GuHCl), widely used in yeast prion research, disrupts prion propagation through effects on the Hsp104 chaperone system [2,3]. Here, we evaluated how GuHCl presence modulates transcriptional stress outputs across three prion phenotypes: [*psi*$^-$], weak [*PSI*$^+$], and strong [*PSI*$^+$].

Cells were challenged with medium and extreme heat shock, and with oxidative stress using 1 mM and 3 mM H_2O_2. Transcript levels of the heat-shock marker *UBI4* [4] and oxidative-stress marker *TRX2* [5] were quantified by RT-qPCR. *MEX67* was used as the reference gene based on stable expression across all stress conditions. GuHCl effects were expressed as relative quantification (RQ) compared with matched −GuHCl controls for each phenotype and stress intensity.

Under heat shock, GuHCl decreased *UBI4* in [*psi*$^-$] and strong [*PSI*$^+$] at both intensities, with mean RQ values of 0.37 and 0.12 in [*psi*$^-$], and 0.25 and 0.079 in strong [*PSI*$^+$]. The direction of change was consistent across biological replicates, although effect sizes varied. In weak [*PSI*$^+$], GuHCl increased *UBI4* with mean RQ values of 3.75 and 2.16, but replicate consistency was lower, indicating greater biological variability. Under oxidative stress, GuHCl increased *TRX2* across all phenotypes at both 1 mM and 3 mM H_2O_2. Mean RQ values at 1 mM were 4.91 in [*psi*$^-$], 7.43 in weak [*PSI*$^+$], and 14.46 in strong [*PSI*$^+$]. At 3 mM, mean RQ values were 6.79 in [*psi*$^-$], 8.86 in weak [*PSI*$^+$], and 10.11 in strong [*PSI*$^+$]. Replicate direction was consistent across oxidative-stress conditions, with the strongest replicate agreement observed for strong [*PSI*$^+$] at 1 mM H_2O_2.

Together, these data show that GuHCl can either dampen or enhance stress-gene transcription depending on stress modality and prion variant state, with oxidative-stress induction of *TRX2* showing higher replicate consistency than heat-shock modulation of *UBI4*.

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Antibacterial activity of formulations based on thyme and coriander essential oils against *Escherichia coli* and *Staphylococcus aureus*

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Infections caused by opportunistic bacteria such as *Escherichia coli* and *Staphylococcus aureus* constitute a significant global public health concern. *E. coli* is a Gram-negative rod that forms part of the normal human intestinal microbiota; however, pathogenic strains (including EHEC, EPEC, and UPEC) can cause gastrointestinal infections, urinary tract infections, sepsis, and meningitis. *S. aureus* is a Gram-positive coccus that colonizes the skin and mucous membranes and is responsible for skin and soft tissue infections, pneumonia, bacteremia, and toxic shock syndrome. A major clinical challenge is the increasing prevalence of antimicrobial resistance, including methicillin-resistant *S. aureus* (MRSA) and *E. coli* strains producing extended-spectrum β-lactamases (ESBLs) [1-2].

Both bacteria are capable of surviving on environmental surfaces, such as elements of public infrastructure (playgrounds, benches, handrails), thereby increasing the risk of exposure, particularly among children. Consequently, there is growing interest in identifying effective and safe alternatives to conventional disinfectants. Biopreparations based on natural bioactive compounds with antimicrobial activity are attracting growing scientific interest.

The aim of the study was to evaluate the antibacterial activity of three essential oil-based formulations against *E. coli* and *S. aureus*. Three compositions were developed using thyme and coriander essential oils as the base, combined with selected oils: eucalyptus and lemon (Formulation 1), sage and marjoram (Formulation 2), and bergamot and peppermint (Formulation 3).

Antibacterial activity was assessed using the agar well diffusion method with reference strains of *E. coli* and *S. aureus*. A volume of 20 µL of each tested formulation was applied into wells prepared in Mueller-Hinton agar. Polysorbate 20 and ethanol served as negative controls, while the antibiotics tetracycline (TE) and ciprofloxacin (CIP) were used as positive controls. After incubation, the diameters of growth inhibition zones were measured. Additionally, the bactericidal activity of the formulations was investigated.

All tested compositions demonstrated bacteriostatic activity against both reference strains. The obtained results indicate that the investigated essential oil formulations may serve as a potential basis for the development of natural disinfectant preparations intended for application on frequently touched surfaces.

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Effects of acetamiprid on the metabolic activity of *Hirsutella* spp.

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Entomopathogenic fungi (EPF) are increasingly recognized as promising alternatives to chemical insecticides due to their natural ability to infect and eliminate insect pests. Their incorporation into integrated pest management (IPM) programs may contribute to reducing the use of synthetic pesticides and mitigating their negative environmental impacts. Among EPF, *Hirsutella* spp. represent a relatively understudied group, highlighting a significant research gap. Data regarding the tolerance of this genus to commonly used insecticides, including neonicotinoids such as acetamiprid, remain limited.

The aim of the present study was to evaluate the effect of acetamiprid on the metabolic activity of two *Hirsutella* spp. strains. Conidial suspensions (10^4 spores/mL) were exposed to acetamiprid at concentrations ranging from 0.16 to 80 mg/L in 96-well microplates. The plates were subsequently incubated at room temperature for 3 and 5 days depending on the strain. Following incubation, 0.1 M phosphate buffer containing fluorescein diacetate (FDA) was added to each well. Fluorescence intensity was measured using a spectrofluorometer.

The results demonstrated that acetamiprid exerted only a minor effect on the metabolic activity of the tested strains. Both strains exhibited comparable responses to the insecticide. A significant reduction in metabolic activity (approximately 60%) was observed only at the highest tested concentration (80 mg/L), whereas lower concentrations caused slight or statistically non-significant inhibition.

These findings indicate that the tested *Hirsutella* spp. strains exhibit considerable tolerance to acetamiprid, maintaining metabolic activity even under relatively high levels of exposure to this insecticide. This characteristic may enhance their potential for incorporation into integrated pest management strategies, particularly in environments where neonicotinoid residues are present.

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Impact of *Priestia megaterium* on the Glucosinolate Composition of Radish Microgreens

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Radish microgreens (*Raphanus sativus*) are valued for their high concentration of health-promoting secondary metabolites, particularly glucosinolates (GSLs). These sulfur-containing compounds are key precursors to bioactive isothiocyanates, which exhibit potent antioxidant and anticancer activities in the human body. Plant Growth-Promoting Rhizobacteria (PGPR), such as *Priestia megaterium*, are often utilized to promote plant growth and modulate plant metabolic pathways.

The aim of this study was to determine whether inoculation with *P. megaterium* can affect the GSL content in radish microgreens. Three experimental variants were established: a control group, substrate inoculation (coconut coir inoculated with bacteria) and seed inoculation (seeds coated with a bacterial suspension prior to sowing). Following the growth period, metabolites were extracted using the modified Matyash method and subsequently analyzed via ultra-high-performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC-HRMS/MS).

Several key glucosinolates were successfully identified across all samples, including glucoberteroin, glucoraphanin, glucobrassicin, glucoerucin, and glucoiberverin. However, comparative analysis (one-way ANOVA) revealed no statistically significant differences ($p > 0.05$) in the concentrations of these compounds between the control and the two inoculated groups.

Our previous studies have shown that the presence of *Priestia megaterium* positively influences the growth of radish microgreens. The present results indicate that, under the tested experimental conditions, bacterial inoculation regardless of the application method does not significantly affect the glucosinolate biosynthetic pathway. These findings suggest that *P. megaterium* can promote plant growth without compromising the accumulation of health promoting glucosinolates in *Raphanus sativus* microgreens. This highlights the potential of this bacterium as a beneficial bioinoculant for improving microgreen production while maintaining their nutritional quality.

Acknowledgments

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How *Trichoderma* degrades PBAT: the pivotal role of extracellular enzymes

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The growing use of poly(butylene adipate-co-terephthalate) (PBAT) in agriculture increases the need to understand how this material is broken down in soil. In this study, we investigated whether *Trichoderma harzianum* KKP534 can degrade PBAT microparticles and focused on the role of extracellular enzymes in this process.

Scanning electron microscopy (SEM) showed clear changes on the surface of PBAT after incubation with the fungus. The polymer exhibited cracks, erosion, and strong mycelial attachment, indicating active interaction between fungal hyphae and the plastic surface. Proteomic analysis of the fungal secretome, with the use of LC-MS/MS technique, revealed a wide range of extracellular proteins potentially involved in polymer degradation. Among them were hydrolases, such as cutinase- and protease-like enzymes. The presence and differential abundance of these proteins in PBAT-treated samples suggests that *T. harzianum* utilizes a coordinated set of secreted enzymes to initiate and support the degradation of polymers into fragments that can be further degraded by intracellular enzyme systems (e.g., cytochrome P450).

These results highlight the importance of extracellular enzymes in fungal degradation of biodegradable plastics and provide insight into how soil fungi may contribute to the transformation of PBAT in the environment.

Acknowledgments

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Halophilic archaea from the Bochnia Salt Mine air induces trained immune tolerance in THP-1 cells

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Trained immunity is a process of functional and epigenetic reprogramming of innate immune cells, such as monocytes and macrophages. This phenomenon allows these cells to "remember" previous encounters and respond more effectively to subsequent pathogenic or inflammatory challenges [1]. So far, no studies were done to investigate the capacity of extremophilic microorganisms to induce trained immunity. Halophilic archaea, which belong to extremophiles, were demonstrated to activate human dendritic cells and orient T helper cell responses, which may be important for the therapeutic effects of salt mine air exposure during halotherapy [2]. However, it remains unknown whether extremophiles may induce trained immunity and what would be the impact of this induction.

Thus, the aim of this study was to determine the ability of halophilic archaea to induce trained immunity in the THP-1 monocytic cell model. The cells were stimulated for 6h and restimulated after 5 days with the halophilic archaeal strains isolated from the air of the halotherapy zone in the Bochnia Salt Mine. The anti-inflammatory IL-10 and the pro-inflammatory TNF-α cytokines were determined by ELISA in the culture supernatants collected after 6 hours, 5 days and 24 hours restimulation.

The results demonstrated for the first time that halophilic archaea isolated from the air induce the production of IL-10 and TNF-a by THP-1 cells. Moreover, the induction of immunological "tolerance" was observed, suggesting that halophilic archaea from the Bochnia Salt Mine may play an important role in modulating inflammatory processes.

Acknowledgments

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Isolation and characterisation of beneficial bacteria from heat stress environment on coal-waste dump

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In the era of global warming, heat stress is becoming one of the key factors limiting proper plant functioning. Beneficial plant-associated microorganisms, supportplant nutrition, physiology, and growth in harsh environments and help them survive even when the temperature is outside the optimal range for plant growth. Plants thriving in extreme ground temperatures caused by underground coal waste combustion represent a fascinating case study, given the challenging conditions they face. One of the possible mechanisms underlying improved plant growth under heat stress conditions is the presence of beneficial microorganisms, which have plant growth-promoting (PGP) properties, enhancing tolerance to elevated temperatures even up to 40°C. Therefore, considering areas with varying ground temperaturesand the plants growing there, the main objective of this project was to isolate and characterise beneficial bacteria (endophytes and rhizospheric) with PGP mechanisms from plant tissues and rhizosphere soil.

In the present study, 117 bacterial strains were isolated from plant tissues (roots, leaves) and rhizospheric soil of two plant species: white fleabane (*Erigeonannus*) and bird's-foot trefoil (*Lotus corniculatus*), growing on a coal-waste dump with different ground temperatures (25, 28, 35, 41°C). All isolates were characterisedwith PGP mechanisms, including their ability to grow under osmotic stress conditions, production of auxin (IAA), antioxidant enzymes, ammonia, phosphate solubilization, and atmospheric nitrogen fixation. The highest average values of phosphate solubilization capacity were observed in bacterial strains isolated from ground temperature 28°C and 35°C (191% solubilization efficiency (SE), 2.59 phosphate solubilization index (PSI)). About 70% of the isolated strains were capable of growing under high salt concentrations, with this trait being most frequently observed among strains isolated from plants growing at 35°C. The ability to fix nitrogen was detected in all isolates from 28°C and 35°C. Moreover, bacterial antioxidant enzyme activity was highest in bacteria isolated from 25°C and decreased with increasing temperature gradient. All isolated strains demonstrated the ability to produce IAA in the range from 2.29 µg/ml to 31.68 µg/ml. The lowest ability to IAA production was observed in the endophytic bacteria isolated from plants growing in the 41°C, while the highest production was observed in the endophytes isolatedfrom plants growing in the 35°C ground temperature.

The obtained results suggest that the presence of bacterial plant growth-promoting mechanisms depended on the ground temperature. The expression of PGP mechanisms in beneficial microorganisms may be associated with the early response of the microbiome to changing environmental conditions, which may consequently support plant development and adaptation. The findings of this study provide valuable insights into temperature dependent functioning of plant- beneficial bacteria. The collection of well-characterised microorganisms may contribute to advancing the development of microbial-based strategies for improving crop resilience under changing climatic conditions.

Biochemical and Genetic Characterization of an Environmental *Pantoea agglomerans* Strain with Evaluation of Its Application Potential

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Pantoea agglomerans is a widespread environmental bacterium commonly associated with plants, soil, and water ecosystems. Due to its metabolic versatility, selected strains of *P. agglomerans* have attracted increasing interest as potential plant growth–promoting bacteria (PGPB) and biological control agents. However, the functional properties and genetic background of many environmental isolates remain insufficiently characterized. In this study, an environmental strain of *Pantoeaagglomerans,* isolated from *Orobanche picridis* was subjected to comprehensive biochemical and genetic characterization in order to assess its potential applications. Phenotypic analyses included the evaluation of metabolic activity, safety-hazard issues, enzymatic properties, motility, biofilm formation, and selected antagonistic traits against other microorganisms. The strain's biochemical profile was determined using standard microbiological and biochemical assays. Genetic characterization was performed using whole-genome sequencing in long-read technology, followed by bioinformatic analysis, including genome annotation and identification of genes potentially involved in plant–microbe interactions, stress resistance, and antimicrobial activity. Particular attention was given to gene clusters associated with secondary metabolite production, motility and traits relevant to plant growth promotion. The obtained results indicate that the analyzed *P. agglomerans* strain exhibits features characteristic of environmentally adapted bacteria with promising biotechnological potential. The combination of biochemical properties and genetic determinants suggests possible future applications of the strain as a plant growth promoter and/or a biological control agent in agriculture. Further functional and field-based studies are required to confirm its practical usefulness.

Selection and Characterization of PGPR Microorganisms for Phytoremediation Support

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Phytoremediation is an environmentally friendly and promising approach for the remediation of soils contaminated with toxic substances, including heavy metals and organic pollutants. The efficiency of this process can be significantly enhanced through the application of plant growth-promoting microorganisms (PGPR) and specialized microbial consortia. The aim of this study was to evaluate the potential of a selected microbial consortium as a biostimulant supporting phytoremediation processes.

Microorganisms were isolated and characterized, followed by the selection of strains exhibiting PGPR traits. The screening was based on their ability to produce indole-3-acetic acid (IAA), synthesize siderophores, and solubilize phosphates. Based on these characteristics, a microbial consortium was developed with the potential to promote plant growth and increase the bioavailability of nutrients and contaminants. Preliminary findings indicate that the application of the microbial consortium may improve plant performance and enhance phytoremediation efficiency. Currently, in vitro experiments using plant models are being conducted to assess the effects of the developed biostimulant on plant growth and their capacity for pollutant accumulation and/or degradation.

The obtained results suggest that integrating phytoremediation with microbial biostimulation represents a promising strategy for the reclamation of degraded environments and sustainable soil management.

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The influence of reproductive tract microbiota on the efficacy of chemotherapy in female upper reproductive tract cancers

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Introduction. The human microbiota is increasingly recognized as a modulator of cancer progression and response to treatment. In gynecological oncology, the composition of the vaginal and uterine microbiome may significantly influence the microenvironment of tumors such as endometrial and ovarian cancers. Understanding how specific bacterial strains interact with cancerous cells during treatment is essential for developing personalized supportive therapies.

Objectives. This study aims to evaluate the impact of different bacterial species, including beneficial *Lactobacillus* strains and potential pathogens, on the survival and proliferation of female reproductive tract cancer cells subjected to chemotherapy.

Materials and Methods. The study utilized the Ishikawa (endometrial cancer) and OVCAR-8 (ovarian cancer) cell lines. A clonogenic assay was performed to assess long-term cell viability and colony-forming ability. Cells were treated with a *Lactobacillus* cocktail or individual pathogenic strains, including *Anaerococcus vaginalis*, *Neisseria gonorrhoeae*, and *Gardnerella vaginalis*, in the presence of chemotherapeutic agents.

Results. Preliminary results for the Ishikawa line revealed significant differences in cell proliferation depending on the microbial environment. The *Lactobacillus* cocktail significantly reduced the survival of cancer cells compared to the cells treated with pathogenic bacteria. Conversely, the presence of *A. vaginalis*, *N. gonorrhoeae*, and *G. vaginalis* resulted in higher cell survival rates. Ongoing experiments with the OVCAR-8 line suggest a similar trend in the ovarian cancer model.

Conclusions. The presence of *Lactobacillus* species in the tumor microenvironment may enhance the efficacy of chemotherapy, whereas common reproductive tract pathogens appear to exert a risk of worse chemotherapy outcomes. These findings suggest that the microbiota composition is a crucial factor in oncological outcomes and could serve as a potential target for improving chemotherapy response in patients with gynecological cancers.

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Bioreactor scale-up of *Pleurotus ostreatus* mycelium for sustainable biomass production

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Production of fungal mycelium via submerged fermentation (SmF) has gained increasing attention as a sustainable approach for producing high-value biomass for food, feed, and biotechnological applications. Although *Pleurotus ostreatus* has been widely studied in submerged cultures at laboratory micro-scale, the successful transfer of these cultivation systems to larger, controlled bioreactor environments remains a key technological challenge. The primary objective of this study was to evaluate the feasibility of scaling up submerged cultivation of *Pleurotus ostreatus* from micro-scale conditions to macro-scale operation in a stirred-tank bioreactor.

Cultivation was carried out in a laboratory-scale stirred-tank bioreactor with a working volume of 3 L under controlled process conditions. The fungus was grown in a defined liquid medium composed of water, glucose, and essential microelements. Fermentation parameters were maintained at a constant temperature of 25 °C and pH 5.5, ensuring stable growth conditions suitable for scale-up studies.

The structural organization and morphology of the produced mycelium were examined using scanning electron microscopy (SEM), enabling detailed visualization of hyphal networks, surface characteristics, and growth forms developed under submerged conditions. In addition, chemical analysis of the harvested mycelial biomass was performed to characterize its composition, with particular emphasis on components relevant for industrial and biotechnological applications.

The results confirm that *Pleurotus ostreatus* can be effectively cultivated under macro-scale submerged fermentation conditions while preserving favorable morphological and compositional characteristics. This study demonstrates that the transition from micro-scale cultures to controlled bioreactor systems is achievable and provides a basis for further process optimization and industrial-scale implementation of submerged *Pleurotus ostreatus* mycelium production.

Keywords: *Pleurotus ostreatus*, submerged fermentation, bioreactor, mycelial biomass

Insights into the genomic diversity of *Proteus mirabilis* serogroup O78 and the O-antigen biosynthesis cluster genes

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Proteus mirabilis is an opportunistic pathogen frequently associated with urinary tract infections, where its O-antigen plays a key role in immune evasion and host interaction. Despite the clinical relevance of serogroup O78, its genomic diversity and the organization of O-antigen biosynthesis genes remain poorly characterized. In this study, we analyzed 20 *P. mirabilis* O78 isolates to explore their genomic variability and the structure of the O-antigen gene cluster. Genomic DNA was isolated and sequenced, followed by comparative analyses and phylogenetic reconstruction to assess relationships among the strains. We identified the O-antigen biosynthesis clusters and compared their genetic composition, revealing conserved core regions alongside notable variability, particularly in the genes encoding DUF1919-domain-containing proteins, a conserved protein family (PF08942) whose function remains unknown. These findings provide new insights into the genetic architecture of the O78 serogroup and highlight potential molecular markers relevant for serotyping, epidemiological studies, and understanding O-antigen evolution in *P. mirabilis*.

Caspase-4-dependent EV Release in Human Coronary Endothelial Cells – A Proteomic Analysis

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Atherosclerosis is an underlying cause of various cardiovascular diseases. It is a slowly developing process of formation of inflammation foci - "fatty streaks" - in the blood vessel. Inflammatory process has critical role in pathogenesis and development of atherosclerosis and it is driven by multiple cells and factors. Human coronary artery endothelial cells (HCAECs) can produce inflammatory mediators such as interleukin-18 (IL-18) and express caspase-4, a component of the non-canonical inflammasome [1]. Lipopolysaccharide (LPS), which may enter the bloodstream under conditions of increased intestinal permeability (e.g. "leaky gut"), can get access to the cytosol of endothelial cells and activate non-canonical inflammasome, causing cell death.

We demonstrate that in HCAECs caspase-4 activation by LPS transfection (but not extracellular LPS)induces the secretion of large numbers of extracellular vesicles (EVs) with immunomodulatory properties. Secretion of EV is strictly dependent on caspase-4 and precedes pyroptosis.We applied quantitative proteomics combined with bioinformatics to investigate the EV-mediated protein secretion process, identifying and quantifying more than 3000 EV-associated proteins. Bioinformatics-assisted analyses point to Rac1 GTPase as a regulator of EV secretion. Rac1 GTPase is a key regulator of cytoskeleton-dependent signaling pathways controlling endothelial migration, proliferation and vascular homeostasis, and has also been associated with oxidative stress regulation [2]. However, the mechanisms linking intracellular LPS sensing with EVs release in coronary endothelial cells remain poorly understood and the role of Rac1 in LPS-induced release from coronary endothelial cells remains unclear. Our data indicate that Rac1 regulates the inflammatory process in HCAECs affecting both the IL18/LDH release and EV secretion.

In atherogenesis, endothelial dysfunction is followed by macrophage activation and inflammation. We hypothesized that upon LPS transfection HCAEC secrete EV which modulate macrophages. We have stimulated human monocyte-derived macrophages with EV isolated from mock- and LPS-transfected HCAEC. Our data indicate that expression of several inflammation-related genes is differentially regulated, what may be associated with atherogenesis. These findings highlight Rac1-dependent EV secretion as a potential mechanism of endothelial–macrophage crosstalk during inflammatory processes associated with atherosclerosis.

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ABSTRACTS:

BIOMEDICAL ENGINEERING AND MOLECULAR MEDICINE

Impact of surface enhanced flake graphite properties on a cathode performance in MFCs

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In microbial fuel cells (MFCs), cathode material is one of the crucial elements, facilitating the oxygen reduction reaction (ORR). Due to oxygen mass transfer limitations, biofouling and the degradation of the cathode, current studies focus on balancing durability, cost effectiveness and performance of cathode materials for genuine applications such as the treatment of wastewater with simultaneous power generation which could bring environmental and economical benefits [1].

Among various cathode materials used in air-cathode MFCs, carbon materials are commonly used to their sufficient catalytic properties, high surface area, low cost and feasibility of modifications. Despite the availability of more efficient cathode materials, such as platin, their practical use is limited by poor stability in wastewater conditions or high cost [2].

In this study, five different graphite materials and one graphene oxide material were used to build the air cathode in MFCs and to assess their catalytic performance. Activated carbon CWZ-22 was used as a well-known and highly efficient air-cathode reference material [2]. Catalytic activity and stability of cathode materials were compared by performing polarization experiments periodically throughout the entire study. TGA with FTIR analysis of the initial, as well as after MFC cathode materials were performed to examine their thermal behavior. Furthermore, surface area and pore size distribution of cathode materials were characterized using nitrogen adsorption.

The study revealed the feasibility to employ the surface enhanced flake graphite as the efficient cathode material. Nevertheless, the achieved results did not exceed standard activated carbon. The most efficient graphite material reached 7.54 W/m3, while the CWZ-22 reached 15.05 W/m3, after 5 months of operation. The remaining graphites performance reached between 5.25 and 7.38 W/m3. The active surface area of best performing surface enhanced graphite was more than 5 times lower than CWZ-22 which was identified as a critical factor impacting the electrode performance.

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Asynchronous Processing Architecture and Microservices for High-Fidelity Sleep Quality Monitoring

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Remote sleep quality monitoring requires systems capable of managing massive data volumes without compromising signal integrity or user experience. This work presents the use of wearables with a software architecture designed to capture and process signals through an optimized data flow that guarantees information integrity for recording sessions averaging 8 hours [1].

The ecosystem employs a chest sensor (Suunto Movesense) that transmits raw data via Bluetooth Low Energy (BLE) to our native mobile application. To guarantee the continuity of data acquisition against Android's power optimization policies, a persistent Foreground Service and an abstraction layer (MDS) were implemented to ensure reliable and sequential communication. Data transfer to the server is performed through a chunking protocol over HTTPS/REST, ensuring resilience in the transmission of large data volumes by fragmenting files exceeding 100MB to allow automatic retries and verify integrity via checksums.

The core of the innovation lies in its microservices architecture (Docker) [2] and its data processing capacity. The system decouples data ingestion from inference using a Redis message queue and Celery asynchronous workers. This configuration allows Artificial Intelligence models (LSTM) to achieve exceptional processing times, ranging around 0.6 seconds per hour of recording [3]. The results, including the Apnea-Hypopnea Index (AHI) and severity levels, are synchronized in real-time with a web dashboard (Django) and the mobile App, providing a highly scalable, clinical-grade tool for home monitoring.

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Glycolipids as factors modulating the virulence and biofilm of pathogens – a new approach to infection control

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In light of two significant contemporary challenges in microbiology and biotechnology, there is an urgent need to explore alternative strategies to limit pathogen colonization and reduce virulence. The increasing antibiotic resistance exhibited by microbial organisms and the growing importance of biofilms as a contributing factor to the pathogenesis of infections necessitate the urgent exploration of novel approaches to combat these challenges. Increasing attention is being paid to biologically derived surface-active compounds and sugar surfactants, which, in addition to their classic antimicrobial activity, have been shown to affect the mechanisms responsible for adhesion, biofilm formation, and the expression of virulence factors. The solution to these issues may be found in biosurfactants, which are glycolipids, such as sophorolipids (SLs) and sucrose fatty acid esters (SP50, SP70). These can be used as tools to limit adhesion, biofilms and the virulence of pathogens.

Sophorolipids in their symmetrical (SL_{SS}) and asymmetrical (SL_{NS}) forms exhibited high anti-adhesive activity, particularly against staphylococci. The inhibition of adhesion ranged from 58.8% to 86.4%. However, in the post-adhesive model, the reduction in already attached cells was found to be between 50% and 73.4%. Most significantly, sophorolipids were also found to be highly effective in the removal of mature biofilms, achieving a reduction in biomass of 60–81% at concentration 2mg/ml, depending on the strain and form of the compound. Furthermore, the combination of sophorolipids with lipases resulted in the near-complete elimination of biofilms (up to 99.9%), without compromising the activity of the enzyme itself. Sucrose fatty acid esters SP50 and SP70 were evaluated in parallel. At a concentration of 1 mg/ml, cell adhesion to the surface was reduced by 80–95%, and the biomass of mature biofilms was reduced by 70–90%, with SP70 typically being more effective than SP50. Molecular analysis revealed a significant reduction in the expression of genes associated with adhesion, biofilm, and stress response in *Enterococcus faecalis* (esp, efaA, ctsR) and genes responsible for fimbriae formation, metabolic adaptation, and membrane protein function in *Klebsiella pneumoniae* (mrkA, arcB, ompK35). In the case of *Candida albicans*, a marked reduction in the expression of morphogenesis and pathogenicity genes (HWP1, EAP1, EGF1, ECE1, SAP5) was observed, accompanied by inhibition of hyphal formation. Concurrently, SP50 and SP70 exhibited no evidence of toxicity towards human skin fibroblasts or colon epithelial cells, and effectively restricted the adhesion of *C. albicans* to host cells.

The obtained results indicate that both sophorolipids and sucrose fatty acid esters act primarily as potent modulators of adhesion, biofilm, and virulence factor expression, rather than as classical biocidal agents. It is evident that these compounds represent a promising group of safe biotechnological tools for controlling biofilm-associated colonization and infections.

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Chemopreventive potential of *Aronia melanocarpa* leaf extract and its flaxseed polysaccharide-based encapsulated form in colorectal cancer

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Colorectal cancer (CRC) is one of the most prevalent cancer types. This disease represents the second leading cause of cancer-related deaths worldwide. Continuous development of novel, successful therapies with lower toxicity is still the highest priority widely undertaken in oncology. Recently, there has been particular interest in compounds of natural origin, for instance form *Aronia melanocarpa* leaves, due to their potential preventive and therapeutic effects in combating CRC [1]. Besides utilizing natural extracts directly, an interesting way to enhance their stability, extend circulation time, and improve targeted administration with less harm to healthy cells is to encapsulate them in a carrier [2].

This study aimed to evaluate the effects of aronia leaf extract (ALE), its encapsulated form (EALE), and the carrier itself (flaxseed polysaccharides [UL5.1], FP) on the viability of SW-480 colon cancer cells and their influence on cell death mechanisms. The antioxidant capacity and total polyphenol content of the obtained preparations were also assessed. The impact of ALE, EALE, and FP on the cytotoxicity of SW-480 cells was examined using MTT assays conducted after 24, 48, and 72 hours. Each compound was tested across a wide concentration range of 100–1500 µg/ml. As a positive control 5-fluorouracil (50 and 100µM), a common anticancer drug, was used. Based on the MTT results, two concentrations (200 and 400 µg/ml) were chosen for further Western blot analysis to examine the expression levels of key proteins involved in autophagy (LC3B and p62) and apoptosis (procaspase-3).

Both ALE and EALE significantly reduced CRC cell viability, with similar IC50 values for ALE (383 µg/mL) and EALE (394 µg/mL). Moreover, the cytotoxic effect increased with longer incubation times. Conversely, at all-time points and concentrations, FP showed no or only a slight effect, not exceeding 10% of the control. Analysis of the composition revealed that ALE had the highest total phenolic content (9123.98 mg GAE/100 g), EALE had the second-highest (57773.05 mg GAE/100 g), and FP had the lowest (242.90 mg GAE/100 g). Antioxidant testing further confirmed that ALE had the greatest free radical-scavenging capacity (84726.63 µM TE/100 g), while EALE (50878.39 µM TE/100 g) and FP had the lowest (5525.27 µM TE/100 g). Western blot analysis showed that treatment with ALE, EALE, and FP increased LC3B protein levels in a concentration-dependent manner compared to the untreated control. This effect was most pronounced for ALE. Similar trends were observed with p62 expression: higher levels than the control, but decreasing as the agent concentration increased. Additionally, each agent tested reduced procaspase-3 levels.

To sum up, our results show that both ALE and EALE, but not FP, exhibit cytotoxicity in the SW-480 cell line. It indicates that the carrier does not inhibit ALE's properties. The high polyphenol content and the substantial antioxidant potential of ALE and EALE also suggest that they may possess chemopreventive properties. Furthermore, it can be concluded that autophagy may be involved in cell death, as evidenced by increased LC3B expression. But, in the classical autophagy pathway, increased expression of LC3B should be accompanied by decreased expression of p62. Our findings may suggest a blockade at a stage of autophagy—autophagosome degradation. The decreased expression of procaspase-3, in turn, suggests that cell death may be associated with apoptosis. Therefore, further, more detailed studies are necessary to confirm the exact mechanisms.

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Effects of Ethanol on Safety and Efficacy of Pharmacotherapy

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Ethanol exhibits a particularly complex interaction profile with medicinal products, as it can simultaneously influence pharmacotherapy at the pharmacodynamic, pharmacokinetic, and pharmaceutical-technological levels. The clinical relevance of these interactions depends primarily on the dose of alcohol, the duration of exposure, and the properties of the co-administered drug. From a pharmacodynamic perspective, ethanol can potentiate the effects of central nervous system depressants, thereby increasing the risk of sedation, impaired coordination, reduced psychomotor performance, and, in severe cases, respiratory depression. Of particular importance are interactions with benzodiazepines, opioids, hypnotics, and certain antihistamines and psychotropic agents. At the pharmacokinetic level, ethanol metabolism plays a key role. The oxidation of ethanol by alcohol dehydrogenase and aldehyde dehydrogenase leads to depletion of NAD^+ and an increased $NADH/NAD^+$ ratio, thereby disrupting cellular redox balance. This shift promotes the reduction of pyruvate to lactate, inhibits gluconeogenesis, and decreases lactate clearance. Additionally, a fraction of ethanol is metabolized via the microsomal ethanol-oxidizing system, including CYP2E1, whose activity is upregulated during chronic alcohol consumption. This may enhance the formation of toxic drug metabolites; for which chronic ethanol exposure may increase the production of the hepatotoxic metabolite NAPQI. Technological interactions are also relevant, particularly the risk of dose dumping resulting from the accelerated release of the active substance from certain modified-release formulations. In summary, ethanol should be regarded as a significant and multifaceted risk factor for therapeutic failure and adverse drug reactions, warranting careful consideration in both clinical practice and patient education.

Cadherin-switch-independent hybrid EMT induced by CHK1 and ALDH inhibition in 3D ovarian cancer spheroids

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Ovarian cancer remains a leading cause of gynecologic cancer mortality, with more than 313,000 new cases and approximately 207,000 deaths annually. Late presentation, heterogeneity, and recurrent chemoresistance demand models that better recapitulate tumor biology and adaptive stress responses. Epithelial-mesenchymal transition (EMT) is central to invasion, dissemination, and resistance; contemporary evidence shows EMT proceeds across a heterogeneous ensemble of hybrid E/M phenotypes that maintain epithelial traits while acquiring mesenchymal features, thereby maximizing plasticity and metastatic potential [1].

To better reflect tumor behavior, OVCAR-8 cells were grown as 3D spheroids, which better mimic microenvironmental cues (cell-cell/ECM interactions, gradients, drug penetration) than 2D systems. Cytotoxicity was quantified using the resazurin reduction assay. Dose selection for downstream assays was based on preliminary combination activity: 5 µM MK-8776 and 2.5 µM disulfiram for TOV-21G; 2.5 µM MK-8776 and 5 µM disulfiram for OVCAR-8.

Western blot analysis in TOV-21G and OVCAR-8 revealed a non-classical EMT response: E-cadherin downregulation (TOV-21G), vimentin upregulation, TIMP2 downregulation, and an absence of N-cadherin increase (with a downward trend), i.e., no cadherin switch. These coordinated changes indicate induction of a partial/hybrid EMT marked by partial remodeling of adhesion and cytoskeleton rather than terminal mesenchymal conversion [2].

In ovarian cancer, CHK1 inhibition (MK-8776) can potentiate cytotoxic stress and rewire DDR programs, while ALDH inhibition (disulfiram) can deplete stem-like, chemoresistant populations - mechanistically aligning with the observed phenotypic shift and cytotoxicity in a 3D context. These findings position EMT plasticity as both a readout and a therapeutic vulnerability, supporting combinations that pressure cancer cells into unstable hybrid states while diminishing survival pathways.

MK-8776 and disulfiram, assessed in 3D OVCAR-8 spheroids and TOV-21G, drive cytotoxicity and a hybrid EMT profile (E-cadherin↓/Vimentin↑/TIMP2↓/no N-cadherin switch). This underscores the value of 3D models and EMT-informed strategies in ovarian cancer.

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Physicochemical Characterization, Biological, and Antioxidant Properties of Chitosan/Quercetin Composites

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Chitosan films enhanced with quercetin represent a highly promising category of biomaterials for applications in wound healing due to their favorable biocompatibility, bioactivity, and antioxidant properties [1]. This study concentrated on developing a variety of chitosan–quercetin composites, including those augmented with metal nanoparticles (Ag, Cu, Bi, and Au), by employing a solvent-casting method. The composites underwent comprehensive physicochemical characterization (FTIR, UV–Vis, and SEM analysis), evaluation of water solubility, mechanical testing, and determination of antioxidant activity through the DPPH assay. Biological assessments included hemolysis testing, hemoglobin adsorption, and cytotoxicity evaluations against skin fibroblasts (BJ) and peripheral blood mononuclear cells (PBMCs). Additionally, cell adhesion was analyzed through DAPI staining, and the protective abilities of the composites were evaluated using a hydrogen peroxide-induced oxidative stress model.

The majority of films exhibited good biocompatibility, leading to minimal hemolysis or cytotoxicity and no DNA damage. This further reinforces their potential as effective biomaterials for wound healing. The inclusion of quercetin and metal nanoparticles led to enhanced antioxidant effectiveness and improved structural integrity of the films under conditions resembling physiological environments. Mechanical analysis revealed that certain formulations demonstrated increased tensile strength and elasticity, which are vital factors for effective wound dressing applications. Furthermore, decreased water solubility suggested enhanced resistance to premature degradation while ensuring an adequate moisture balance at the wound site.

In summary, the results indicate that chitosan films enriched with quercetin, featuring improved mechanical properties and water resistance, serve as a versatile biomaterial platform with significant potential for wound care, although further optimization and translational research are needed to validate their clinical use.

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Electrochemical Approach to Hormone Sensing: Bridging Analytical Challenges and Clinical Diagnostics

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Regular and continuous monitoring of hormone levels plays a key role in biomedical research and the management of therapies, enabling the assessment of endocrine function, and optimization of treatment regimens. However, standard diagnostic approaches still rely on sophisticated, often expensive instrumentation and centralized laboratory testing, which can be time-consuming, economically demanding, and not readily accessible in resource-limited or decentralized settings. Electrochemical sensing presents a promising alternative, offering the possibility of fast, sensitive, and cost-effective measurements with the potential to be integrated into compact, handheld point-of-care devices. A key limitation, however, is the performance of such sensors in real biological matrices, where complex sample composition, fouling, and matrix interferences can strongly affect selectivity, stability, and reproducibility. This project discusses existing electrochemical strategies for hormone detection, evaluates their strengths and limitations in clinically relevant conditions, and is carried out in an academic–industrial collaboration framework, aiming to translate electrochemical hormone sensing from the laboratory level into robust, user-friendly, and reliable point-of-care applications in the future.

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Chiral Imine Coordination Cages as Smart Platforms for Drug Delivery and Enantioselective Recognition

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Coordination-based molecular cages constitute a highly versatile class of supramolecular systems and have attracted considerable attention owing to their broad potential in advanced molecular medicine. [1] These three-dimensional architectures, formed via coordination-driven self-assembly of metal ions and organic ligands, exhibit distinctive structural and functional features that make them particularly well suited for the selective recognition, binding, and transport of molecular species. [2]. Their ability to encapsulate guest molecules within well-defined internal cavities, combined with finely tunable host–guest interactions, provides a robust foundation for the design of highly sensitive and selective molecular platforms. Such systems have demonstrated significant promise in applications including controlled drug delivery, enantiomeric recognition [3], targeted therapeutic strategies.[4]

This presentation focuses on the synthesis and potential applications of chiral coordination cages specifically designed for the precise and selective separation of enantiomers of organic molecules (including pharmaceuticals), as well as for their transport and stabilization. The coordination cages were synthesized using chiral imine ligands (L1) combined with Pd^{2+} ions to afford assemblies with well-defined geometries (C1). [Figure 1] These metallo-supramolecular architectures were comprehensively characterized both in the solid state (SXRD) and in solution (^{1}H NMR, DOSY NMR, and ESI-MS).

In summary, the development of chiral coordination cages represents a promising strategy for advancing molecular medicine technologies. The interplay between precise synthetic control and functional versatility enables the creation of highly selective and efficient platforms for molecular recognition, transport, and therapeutic applications. By focusing on the synthesis and functional exploration of these innovative supramolecular structures, this work contributes to the progress of precision detection and highlights the transformative potential of coordination chemistry in modern medicinal chemistry.

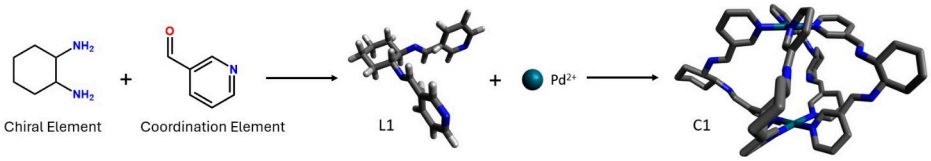

Fig. 1. Scheme of the synthesis of a chiral metallosupramolecular cage based on Pd(II) ions.

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The effect of CHK1 kinase inhibitor and aldehyde dehydrogenase inhibitor on OV90 ovarian cancer cells

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Ovarian cancer is considered one of the most important global challenges in medicine. According to GLOBOCAN 2022 data, ovarian cancer is the eighth most common cancer and the eighth leading cause of death among women worldwide [1]. Metastases to the abdominal peritoneum, among other places, significantly hinder surgical reduction of tumor mass. Chemotherapy for ovarian cancer is usually based on platinum compounds and taxanes. Currently used therapeutic methods do not achieve the intended goal of completely eradicating the cancer [2].

The aim of this study is to investigate the action of drugs targeting the repair mechanism of cancer stem cells. Checkpoint 1 kinase inhibitors (CHK1i) together with disulfiram comprehensively target the cell repair system. Disulfiram, an example of an ALDH1 inhibitor known from alcoholism therapy, causes DNA damage resulting from the accumulation of aldehyde, which leads to the initiation of repair processes through non-homologous end joining (NHEJ) or homologous recombination (HR). In turn, the CHK1 inhibitor (CHKi, MK8776) induces DNA damage by blocking the repair of genetic material that occurs during cell cycle arrest.

The cytotoxicity of disulfiram and MK8776 (CHK1i) was determined in studies using both monotherapy and combination therapy to determine the coefficient of drug interaction (CDI) using the MTT cytotoxicity test. The ability of cells to form colonies was also estimated using the clonal growth method. The studies were conducted on the OV90 line, which exhibits epithelial morphology and was isolated from a patient with malignant serous papillary adenocarcinoma. After applying appropriate concentrations of the tested drugs, a decrease in the viability of OV90 cells was observed in the MTT cytotoxicity test, as well as a decrease in the ability of cells to form colonies. Additionally, the results from both methods indicate that combination therapy is more effective than monotherapy with disulfiram or MK8776. Targeting stem cells and using drug combinations may have beneficial effects and provide valuable information needed to combat the very dangerous adversary that is ovarian cancer and cancer in general.

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3D printed cel for bioelectrochemical applications

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Impedance measurements is commonly used tool for tracking growth of cells in time, construction of sensors or in general provides information about dielectric properties of the electrode surface. This is due to low interference of sample matrix (no addition of markers) and real time response which help e.g. in acquiring information about drugs affecting growth and proliferation of cells, adhesion and migration. Usually, equipment for such analysis has high costs and is not flexible for applying other experiments during measurement. By combining ITO (Indium Tin Oxide) electrodes and Fused Deposition Modelling (FDM) 3D printing we might reduce cost of apparatus with increasing flexibility of such system[1], [2], [3], [4], [5]. Implementation of transparent ITO working electrode (WE) at bottom of electrochemical cell will enable microscopy based cell counting and real-time monitoring of morphological changes, as well as additional staining approaches, e.g., fluorescence based methods. Application of FDM might significantly decrease cost of consumables, usage of different materials might increase reusability of system, and finally 3D printing enables fast prototyping and easy modification of system according to current needs such as modularity or addition of other techniques for quantification of cells.

The aim of my presentation is to show the design of the electrochemical cell for presumptive bioelectrochemical applications.

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Preludium Bis – 2022/47/O/ST4/02557 – cells fabrication.

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Histamine-mediated cytokine production after stimulation with 5-FU loaded PLA-based nanoparticles

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Strategies used by cancer cells to evade the host's immune system mechanisms pose a pivotal challenge in the development of effective therapies. Nowadays, therapeutic strategies for gastric cancer include 5-fluorouracil (5-FU), but the drug alone lacks immunomodulatory potential and does not induce the production of pro-inflammatory cytokines [1]. On the other hand, the use of nanoparticles (NPs) modified with biologically active substances, such as histamine, can potentially enhance the therapeutic effect of 5-FU by inducing biological responses in host cells. The use of histamine (His) as a mediator of immune responses in the tumour microenvironment may exert immunomodulatory effects on gastric cancer cells.

Poly(lactic acid)-based nanoparticles (PLA-based NPs) loaded with 5-fluorouracil (5-FU) and modified with histamine (His) as end groups were physicochemically characterized. The human cell line AGS CRL-1739 was cultured in medium alone or stimulated with different variants of nanoparticles and stimulants alone at 24 h, 48 h, and 72 h. Then, the cellular uptake of the drug and NPs was evaluated using confocal microscopy. The effect of nanoparticles on AGS cells' IL-1β production was evaluated by measuring marker levels after stimulation using an Enzyme-Linked Immunosorbent Assay (ELISA).

Treatment with NPs containing His led to increased IL-1β production after 24 hours of incubation, and this effect was also observed after extended incubation (72 h). Notably, among all tested nanoformulations, the strongest effect was seen with PLA-His-5-FU compared to other variants. No changes in IL-1β were observed in cultures stimulated with 5-FU or NPs 5-FU alone (unpublished data).

Tested 5-FU-loaded, histamine-modified nanoparticles enhance the production of IL-1β in gastric cancer cells *in vitro* more significantly than 5-FU or NPs with 5-FU alone. The findings suggest that histamine is a key mediator of this process. The observed effect of these nanoformulations indicates a potential induction of a proinflammatory environment, which may offer a therapeutic advantage through immune-mediated recognition of gastric cancer cells.

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Bioinformatic Characterization of IGFBP2 as a Driver of EMT Activation in the Glioma Tumor Microenvironment: Pathway Associations and Drug Sensitivity Implications

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Gliomas are the most frequent malignant primary brain tumors and are classified by the World Health Organization into low-grade glioma (LGG) and glioblastoma multiforme (GBM). Insulin-like Growth Factor Binding Protein 2 (*IGFBP2*) is consistently overexpressed across glioma grades and has recently been implicated in tumor microenvironment (TME) remodeling, M2 macrophage polarization, and poor patient prognosis [1]. Despite this, the precise oncogenic pathways driven by IGFBP2 and its relevance to drug resistance in glioma remain incompletely understood.

We performed an integrative bioinformatic analysis of *IGFBP2* across LGG and GBM using three complementary platforms. Single-cell transcriptomic data from two well-annotated GBM cohorts (GSE131928 and GSE84465) were analyzed via TISH2, enabling UMAP-based dissection of IGFBP2 expression across malignant, immune, and stromal cell populations within the TME. Promoter methylation and co-expression patterns were profiled using MEXPRESS [2], and pathway enrichment alongside drug sensitivity analyses were conducted through the Gene Set Cancer Analysis (GSCA) platform [3], integrating pharmacogenomic data from the CTRP and GDSC databases.

IGFBP2 was broadly expressed across TME cell clusters, with notable enrichment in malignant and stromal compartments. Pathway analysis revealed a significant association between IGFBP2 expression and epithelial-to-mesenchymal transition (EMT) activation in LGG (FDR = 0.007), with a consistent trend in GBM (FDR = 0.06). No significant associations were detected with apoptosis, cell cycle, PI3K/AKT, RAS/MAPK, RTK, TSC/mTOR, or DNA damage response pathways in either tumor type. Drug correlation analyses further indicated that elevated *IGFBP2* expression correlates with resistance to selected chemotherapeutic agents across a pan-cancer cohort.

Our findings identify *IGFBP2* as a transcriptionally selective driver of EMT within the glioma TME and highlight its potential as a pharmacogenomic biomarker. These results provide a rationale for future experimental studies targeting *IGFBP2* to improve therapeutic sensitivity in glioma.

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Single Entity Electrochemical Investigation of Soft Nanovesicles at Polarized Liquid-Liquid Interface

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Impact experiments via single entity electrochemistry of bare soft nanovesicles at polarized liquid-liquid interface remain unexplored[1]. Here, we first confirm formation of negatively charged vehicles composed of sodium dodecyl sulfate (SDS) and cholesterol (hereafter referred to as SDS/Chol vesicles), then probe their interaction with micro water||α,α,α-trifluorotoluene (w||TFT) interface. Cryo-electron microscopy (cryo-EM), dynamic light scattering (DLS), zeta potential measurements, and molecular dynamics (MD) simulations were used to characterize size, morphology, surface charge, and molecular insight into SDS/Chol formation. These vesicles produce current transients corresponding to vesicles collisions, and SDS anions transfer to the organic phase. This work offers a new insight for label-free vesicles sensing at soft interfaces.

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Innovative Aggregate Suspension Hydrolysis Method for Characterization of a Novel Bis(2-Hydroxyethyl) Terephthalate Hydrolase GDEst-RM1

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Plastics are widely used polymers prevalent across all sectors of the global economy. However, their extensive use combined with limited natural biodegradation has resulted in rapid accumulation of harmful plastic waste. One promising strategy to address this growing pollution problem is the enzymatic hydrolysis of plastic waste. Over the past few decades, researchers have identified many enzymes capable of degrading polyester-based plastics. One of the most common polyesters, polyethylene terephthalate (PET), has been extensively studied for enzymatic hydrolysis.

During PET biodegradation, various breakdown products can form: bis(2-hydroxyethyl) terephthalate (BHET), mono-2-hydroxyethyl terephthalate (MHET), terephthalic acid (TPA), and ethylene glycol (EG). Certain PET hydrolases only partially degrade the polymer, producing BHET and/or MHET. The accumulation of BHET and MHET slows PET depolymerization because these intermediates can inhibit PET hydrolases. To overcome this limitation, BHET hydrolases can be used to convert these intermediates, thereby promoting more efficient PET depolymerization and enabling complete degradation.

In this study, we developed an innovative quantitative method for the identification and characterization of BHET hydrolases. The approach builds upon a previously described turbidimetric assay of BHET hydrolysis performed in acrylamide gels. The newly developed BHET Aggregate Suspension Hydrolysis (BASH) assay is based on enzymatic degradation of insoluble BHET aggregates, which is monitored by turbidimetric measurements (OD_{600}). The temporal stability of BHET suspensions, as well as their stability across different pH levels and temperatures, was evaluated. The BASH assay was applied to assess the ability of the recombinant microbial esterase GDEst-RM1 to degrade BHET under varying pH and temperature conditions. Furthermore, the results were validated using ultra-high-performance liquid chromatography (UHPLC), which also confirmed MHET and TPA as the degradation products. Preliminary results indicate that GDEst-RM1 can degrade nearly 12.5 mM BHET within 5 minutes at 55 °C, indicating considerable catalytic efficiency

In summary, this study presents the development of the BHET Aggregate Suspension Hydrolysis (BASH) assay and its successful application in the characterization of GDEst-RM1 as a BHET-degrading enzyme. The BASH assay provides a rapid, quantitative, and cost-effective approach for studying BHET hydrolysis, serving as a practical alternative to more advanced techniques such as UHPLC.

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Heterofunctionalized gold nanoparticles as potential siEGFR carriers. Biophysical characterization of complexes.

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This study investigates the interaction between heterofuncionalized gold nanoparticles (AuNPs)[1] and small interfering RNA (siRNA) designed to silence the Epidermal Growth Factor Receptor (EGFR) gene, a common therapeutic target in lung cancer, as well as many others. The therapeutic application of siRNA is limited by significant biological barriers, including rapid renal clearance and enzymatic degradation by plasma RNases. Unmodified siRNA can trigger innate immune responses, while nanoparticle-based carriers often face sequestration by the mononuclear phagocyte system due to protein corona formation. Furthermore, particles exceeding 100 nm are typically eliminated by the liver and spleen. Given siRNA's high molecular weight (~13 kDa) and negative charge, efficient delivery systems are essential to facilitate cellular uptake and ensure endosomal escape, thereby protecting the cargo from lysosomal degradation and enabling cytoplasmic activity[2].

Fluorescence polarisation and gel retardation assay were used to confirm the complexation. Then the AuNPs/siRNA complexes were analysed to determine their physicochemical properties. The hydrodynamic diameter and polydispersity index (PDI) were measured using Dynamic Light Scattering (DLS), while the zeta potential was evaluated via Laser Doppler Velocimetry (LDV). Furthermore, the morphology and structural integrity of the complexes were visualised using transmission Electron Microscopy (TEM)

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Structural and biophysical insights into CHCHD10–TDP-43 interactions in Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive motor neuron degeneration and complex molecular etiology. In approximately 90% of sporadic ALS cases, pathological aggregation and mislocalization of the RNA-binding protein TDP-43 are observed, involving liquid–liquid phase separation [1], aggregation and amyloid formation [2]. More recently, mutations in CHCHD10 have been linked to ALS and related neurodegenerative phenotypes [3], suggesting a potential functional interplay with TDP-43.

In this work, we investigate the interaction between CHCHD10 and TDP-43 and its impact on TDP-43conformational dynamics and aggregation behavior. Using a combination of nuclear magnetic resonance spectroscopy, biophysical aggregation assays, and in silico modeling, we characterize structural and interaction features of CHCHD10 that modulate TDP-43 behavior under aggregation-prone conditions.

Our results indicate that CHCHD10 influences TDP-43 phase behavior and aggregation propensity, supporting a role for CHCHD10 as a modulator of TDP-43 proteinopathy in ALS. These findings provide new mechanistic insight into ALS-associated protein–protein interactions and contribute to a better understanding of how CHCHD10-linked pathways may intersect with TDP-43-driven neurodegeneration.

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Physicochemical properties of chitosan films modified with poly(vinyl alcohol)

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The development of biodegradable polymeric materials is crucial for reducing the negative environmental impact of plastics. Chitosan, as a natural film-forming polymer, is characterized by biocompatibility and broad application potential; however, its limited thermal stability and brittleness restrict its practical use. In this study, chitosan films were modified by incorporating poly(vinyl alcohol) (PVA) in order to enhance their mechanical strength and thermal stability.

The films were analyzed using Fourier transform infrared spectroscopy (FTIR) to confirm the presence of characteristic functional groups of chitosan and to assess their compatibility within the chitosan/PVA blend. The thermal properties of the developed films were determined by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The results clearly indicated that the addition of PVA to the chitosan matrix effectively improves thermal stability and increases the flexibility of the developed materials.

This solution may find applications in environmentally friendly packaging, protective materials, and other systems requiring functional and eco-safe polymeric materials. Such an approach combines favorable biological and mechanical properties with practical application potential in biotechnology and environmental protection, supporting the development of modern sustainable materials.

ABSTRACTS:

ECOLOGY AND ENVIRONMENTAL BIOLOGY

When Nature-based Solutions meet intermittent flow: case study of small urban river

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Urban Nature-based Solutions (NbS) are increasingly implemented as part of climate change adaptation; however, their effectiveness under intermittent flow conditions remains limited and insufficiently documented, particularly in small urban watercourses. Climate change intensifies hydrological variability in cities, leading to periodic drying out of small urban streams and pulse-driven transport of nutrients and suspended solids. This study presents the results of field monitoring of a sedimentation-biofiltration pond (area: 1 621 m^2), implemented on the Łódka River in the city of Łódź, central Poland.

The Łódka River (catchment area: 45 km^2) is characterized by intermittent flow, with water presence mainly during winter and following periods of intense precipitation. Water quality is poor, with average concentration of total phosphorus (TP) 0,6 mg/l, total nitrogen (TN) 3,37 mg/l and total suspended solids (TSS) 0,91 mg/l. Monitoring focused on evaluating changes in water quality during hydrologically active periods, using physicochemical analyses to assess effectiveness of nutrients and suspended solids removal under intermittent flow regimes.

The results indicate that under the observed hydrological conditions (flow in the system was recorded during seven out of the fourteen monitoring expeditions) the sedimentation reservoir was not effective in removing biogenic pollutants or suspended solids. Short flow duration and high flow variability limited effectiveness of sedimentation reservoir, resulting in failure to achieve improvement between inflow and outflow water quality (average concentration increase for TP +28%, TN +108% and TSS +12%). As one of the systems compartments was Permeable Reactive Barriers, we have extended to study to better understand the behavior of calcium-based reactive materials in nutrients removal (the highest phosphate concentration reduction was 95% within 48 hours). We evidenced different efficiency and potential for phosphates remobilization under intermittent flow.

These results demonstrate that the performance of NbS systems in urban environments is strongly constrained by hydrological regime. Evidence from the Łódka River study highlights the need for appropriate design, adequate maintenance strategy, long-term monitoring, and explicit consideration of intermittent flow conditions when implementing systems such as SSBS for urban climate change adaptation. Our assessment of reactive materials provided valuable guidance for adaptating NbS to intermittent flow conditions.

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Fungal community structure in Norway spruce forest soils: eDNA sequencing results from Lithuania

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Environmental DNA (eDNA) sequencing has become a vital tool in mycological research worldwide [1], enabling the identification of thousands of fungal OTUs and broad analysis of community structure [2]. Although forest management effects on fungal sporocarp productivity are relatively well studied, impacts on soil fungal diversity, community composition, and functional traits remain poorly understood [3].

In Lithuania, eDNA methods in mycology are relatively new but rapidly advancing. High-throughput sequencing of soil mycobiomes in Norway spruce forests remains limited, yet available evidence shows these communities have high taxonomic and functional diversity, effectively revealed by eDNA approaches [4].

The aim of this study was to investigate the soil fungal community structure in Norway spruce stands of varying ages in Lithuania using eDNA sequencing, and to assess the impact of forest management intensity on these fungal communities. Soil samples were collected from 83 Norway spruce forest sites in Lithuania, differing in forest management intensity according to the Lithuanian forest classification system, including intensively managed, semi-managed, and natural forests. Fungal community structure was investigated using eDNA sequencing, and taxonomic analysis was performed by comparison with the UNITE database. Thousands of fungal OTUs were identified, encompassing saprotrophic, ectomycorrhizal, and pathogenic ecological groups. The results indicate that the intensity of forest use may influence the structure of soil fungal communities. This study enhances our understanding of the mycobiome in Lithuanian spruce forests and serves as a foundation for further research in ecology, forest management, and biodiversity.

Acknowledgments

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Visualization of silver nanoparticles and microplastics in *Allium cepa* using two-photon confocal microscopy

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Plastic debris gradually breaks down into microplastics (MPs), which are now widely distributed across terrestrial and aquatic ecosystems and are recognised as common environmental contaminants. Due to their high surface area and physicochemical diversity, MPs can adsorb, concentrate, and act as carriers of various pollutants, while their polymer composition plays an important role in determining their environmental fate, bioavailability, and toxicity [1,2]. Under natural conditions, MPs rarely occur alone but are often found with other contaminants, including silver nanoparticles (AgNPs). AgNPs are widely used in consumer and industrial products, are environmentally persistent, and are known to cause surface coating-dependent phytotoxic effects in plants and freshwater algae [3]. Recent studies suggest that interactions between MPs and AgNPs in natural waters may affect AgNPs stability, aggregation, transport, and biological uptake [4]. However, although these contaminants are likely to be present together in the environment, their combined effects on plants are still not well understood. In particular, there is limited information about their simultaneous localisation and possible interaction within root tissues, which makes it difficult to fully evaluate their ecological relevance and potential risks to terrestrial plants. In this study, *Allium cepa* roots were treated for 72 h with fluorescently labelled polystyrene microplastics (PS-MPs) and polymethyl methacrylate microplastics (PMMA-MPs) at concentrations of 40 and 400 mg L^{-1}, applied either alone or in combination with 10.79 mg L^{-1} of laboratory-synthesised AgNPs with two distinct surface coatings, polyvinylpyrrolidone (PVP) and cetyltrimethylammonium bromide (CTAB). Following exposure, roots were thoroughly rinsed and analysed using two-photon excitation microscopy combined with fluorescence-lifetime imaging microscopy (FLIM). This approach enabled the simultaneous detection and spatial mapping of AgNPs and MPs within root tissues. Imaging was performed at both the root surface and the central plane of the root cap and elongation zone. AgNPs were distinguished by their short fluorescence lifetimes (50-100 ps), whereas fluorescent MPs exhibited markedly longer lifetimes (≥3000 ps), allowing reliable separation of both particle types in mixed treatments. AgNP-CTAB showed stronger attachment to the root surface than AgNP-PVP, while PS-MPs displayed higher surface affinity compared to PMMA-MPs. No penetration of either AgNPs or MPs into root tissues was observed. The observed differences in binding behaviour likely reflect the interplay between root structural characteristics and the chemical properties of AgNPs surface coatings and MPs polymer types.

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Back-breaking life of medieval people – investigating occurrence of Schmorl's nodes in two medieval Polish populations

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Schmorl's nodes are herniations of nucleus pulposus (inner part of intervertebral disc) into adjacent vertebral body. Their development is thought to be related to biomechanical stress. Although it remains disputable, many anthropologists claim that they are good markers of physical strain. The aim of this study is to assess occurrence of Schmorl's nodes in two medieval Polish populations: lay urban community from Płock and rural community from Stary Brześć Kujawski (SBK 4) in view to investigate whether patterns of disc herniations reflect differences in their living environment and character of labour.

Sample includes 785 vertebral body surfaces of 12 males and 14 females from Płock and 2658 vertebral body surfaces of 43 males and 45 females from SBK 4. Sex and age at death of individuals were determined according to recommended guidelines [1]. To evaluate occurrence and severity of Schmorl's nodes 3-stages scale was used [2]. To investigate inter- and intrapopulational differences in intensity of disc herniations Chi^2 tests were conducted.

Overall intensity of Schmorl's nodes occurrence is higher in Płock than in SBK 4 (Płock – 23,95% surfaces vs. SBK 4 – 17,19% surfaces; $Chi^2 = 18,17$; $p = 0,0000$). Within both communities males are more affected than females. Results may indicate that daily labour of lay inhabitants of medieval Płock was more physically demanding than labour of farmers from SBK 4. In the other side, differences may reflect biomechanical character of performed movements rather than general level of physical activity. Intersexual variability in intensity of Schmorl's nodes suggests that in both communities males probably performed more strenuous tasks than females. Nevertheless, results should be interpreted with caution as many other factors (stature, body mass, genetics, vertebral morphology) may contribute to development of Schmorl's nodes.

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Micromorphology of seeds and morphology of calcium oxalate crystals as taxonomic features of selected species of *Impatiens* L.

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Advances in molecular methods and SEM have introduced new approaches to species delimitation. In taxonomy, floral characteristics are crucial at all levels of the hierarchy. However, seeds and fruits also show taxonomic significance in some botanical families, including Balsaminaceae. Seed coat micromorphology and seed shape are taxonomically relevant, although they have been comprehensively studied in <200 species of this family. Calcium oxalate crystals, which vary in *Impatiens* tissues, have been described in vegetative and reproductive organs, but their role in seeds—poorly understood—needs to be analysed in terms of consistency and diagnostic value.

Our research included a micromorphological analysis of the seed coat of 12 *Impatiens* species from North America, Europe, and Asia. The aim was to assess the potential usefulness of seed ultrastructural features in the context of taxonomic classification. Initially, a literature review was conducted, which revealed a lack of detailed carpological data on the studied species and a lack of information on the morphology of calcium oxalate crystals in seeds. Biometric measurements and analysis of longitudinal and transverse sections of seeds were performed, taking into account the location of crystal clusters and their density. The analyses were performed using SEM, light microscopy, and confocal microscopy. Microscopic analysis of *Impatiens*seeds revealed considerable morphological variability, confirming interspecific differences in seed coat structure. Calcium oxalate crystals in the form of raphides were also found in species from three continents, indicating a characteristic feature of the genus [1].

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Novel genomic resources and *de-novo* gene identification in two key ecosystem-shaping polychaetes: *Sabellaria spinulosa* and *Hypania invalida*

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Polychaete annelids exhibit extraordinary ecological plasticity and ecosystem-shaping capabilities; however, our understanding of the genomic mechanisms driving their diverse life histories remains limited by a lack of high-quality genomic resources. *Sabellaria spinulosa* (Leuckart, 1849), a subtidal arenaceous biogenic reef-builder, acts as a habitat engineer by constructing complex structures, whereas *Hypania invalida* (Grube, 1860), a tubiculous, Ponto-Caspian invasive species in European waters, thrives in both fresh and brackish waters and tolerates a wide range of salinity, temperature and depth. Both species offer unique natural adaptations to their ecological environment and habitat – yet despite their importance, there are no up-to-date genomic resources available for them. This study tries to address this gap by providing the first near-complete genomic foundations for these polychaetes by providing *de novo* genome, transcriptome and mitogenome assemblies constructed using a hybrid sequencing strategy combining Oxford Nanopore long-reads and Illumina RNA-Seq/ Whole Genome Sequencing (WGS) reads for both species. The *S. spinulosa* genome (842 Mb, ~21.7 kb N50) and the *H. invalida* genome (460 Mb, ~12 kb N50) revealed high levels of heterozygosity (3.6-4.5%) and distinct repeat landscapes. Transcriptome assemblies produced >81,000 transcripts for *S. spinulosa* and >44,000 for *H. invalida*, with completeness scores validated using the *lophotrochozoan_odb12* database through BUSCO. Furthermore, we resolved their mitochondrial architecture, annotating a complete circular mitogenome for *S. spinulosa* and a near-complete (35 genes) fragmented mitogenome for *H. invalida*. The obtained genomic resources were further validated with repeat and functional annotations and integrated with a comparative orthology pipeline against the recently available chromosome-level assembly of the emerging marine annelid model *Capitella teleta*. The functional annotation was performed using a combination of both traditional homology-based approaches and a novel Protein-Language Model (pLM)-based functional annotation. To illuminate the previously unknown gene families, we employed an AI-driven structural annotation framework. This revealed massive, lineage-specific expansions tied to ecological niche colonization. Together, these findings demonstrate how structural proteomics and comparative genomics can decode the evolutionary strategies that shape the life histories of these non-model organisms.

Preliminary development of a reticulocyte staining methodology in blood samples from tits (*Paridae*)

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Reticulocytes (RET) are immature red blood cells. Their count correlates with the erythropoietic activity of the bone marrow. In human and mammalian laboratory medicine, reticulocyte count can be helpful in distinguishing different types of anemia and monitoring the treatment response. In laboratory diagnostics number of reticulocytes (#RET) is one of the parameters of complete blood count. However when it comes to avian blood the topic is scarce and poses methodological challenges.

Due to the fact that mature avian erythrocytes retain a nucleus, it's impossible to use any automated counting methods. In order to count #RET one needs to perform supravital staining with new methylene blue (NMB) and perform manual microscopic counting. This method makes the rRNA remnants visible as dark blue-stained granules inside the cytoplasm. The differences between mammalian and avian erythrocytes make the counting process more difficult in birds, but the main issue is the amount of blood one can take from small passerines. Few studies that do exist were describing bigger species (e.g. chickens or hawks) thus it was possible to sample enough blood to store it in tubes.

In our preliminary studies we had to come up with an idea on how to perform the whole procedure and optimize staining conditions. We can only take a few drops of blood from a single tit. In addition we are usually measuring other parameters. Such a small amount of blood makes it difficult to use any anticoagulant. In that case we have to perform the blood smears as soon as possible during the fieldwork. To stain reticulocytes correctly the blood sample needs to be incubated with new methylene blue for around 15 minutes. Fortunately we discovered that the addition of the stain slows down the process of coagulation and after 15-20 minutes the blood is still viable for smearing.

The next step was to choose optimal time of incubation and ratio of the blood to the stain. After some trial-and-error approach, supported by literature findings we did establish that the incubation should last 15 minutes, and the best ratio is 3 drops of NMB per 1 drop of blood. The slides examined under a microscope show promising results. The RNA remnants are clearly visible. Further studies will focus on the collecting of the samples, counting #RET in smears and analyzing the possible changes.

DNA barcoding of Polish Diplopoda: a reference library and the national checklist

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DNA barcoding is a highly efficient approach for species identification, complementing traditional taxonomic and phylogenetic research while also serving as a crucial tool in biomonitoring and revealing hidden diversity. Methods such as DNA barcoding, metabarcoding, and environmental DNA (eDNA) analysis are widely employed in biodiversity conservation, tracking invasive species, and examining trophic interactions. To conduct this type of research effectively, a reliable DNA barcode reference library is essential, built on high-quality genetic sequences and precise taxonomic determinations. Here, we present the preliminary outcomes of a broader DNA barcoding project focused on Polish diplopods (Myriapoda: Diplopoda), providing an updated checklist of species recorded in Poland together with a reference barcode dataset. We report data from 981 collected and identified specimens, 468 of which yielded COI sequences. Furthermore, we discuss future research perspectives, potential cryptic diversity, and changes in the national fauna, including species newly documented for Poland. Our results underscore the importance of continuous monitoring and molecular tools in tracking biodiversity dynamics.

Safeguarding genetic diversity for *Lynx lynx* conservation – the utility of captive-born individuals and genetic monitoring of reintroduced and native populations

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Conservation of endangered species requires a holistic approach that goes beyond general population reinforcement and includes careful, individual-based management of genetic diversity. As a keystone species and top predator, the Eurasian lynx *Lynx lynx* plays a pivotal ecological role in maintaining the balance of forest ecosystems [1] across its native range throughout Eurasia. After extinctions of this species at the western margins of its range, several reintroduction and translocation programs were implemented to restore viable populations. Despite their return to human-dominated environments in Europe [2], they still face serious challenges, mainly due to genetic factors and human-induced changes in their habitats, leading to population isolation and decline. Usually small and isolated reintroduced populations are vulnerable to inbreeding, which lowers genetic diversity, elevates homozygosity levels, and compromises the adaptability and overall fitness of individuals. A decrease in variability often results in a loss of evolutionary potential in small and isolated populations, which, in the face of habitat fragmentation and climate change, can accelerate the process of species extinction [3]. Maintenance of genetic diversity is, thus, an integral component of conservation planning.

Both adaptive and neutral genetic variation are observed in large predator populations. Adaptive variation refers to genetic differences that increase survival and reproduction in specific environments and is typically sought in genes encoding important life processes. Neutral variation, on the other hand, includes changes that do not directly affect fitness but can provide insight into evolutionary history and population structure and detect whether individuals are inbred (i.e., derived from related mating). Despite their ability to travel long distances during their lifetime, large predators often show genetic differences even between geographically close populations [4, 5, 6], most likely due to isolation. Consequently, assessing neutral genetic variation is essential for understanding population structure and inbreeding levels, particularly in conservation and reintroduction contexts.

Microsatellites, as highly polymorphic neutral markers, are therefore well suited for determining genetic diversity and detecting inbred individuals, thereby providing necessary information for conservation decision-makers. We used 20 microsatellite loci to assess genetic polymorphism and individual inbreeding of captive-born *Lynx lynx* and compared these results to those from wild populations. We thus provide new, individual-based management recommendations for the reinforcement of the reintroduced *Lynx lynx* populations using captive-born individuals. By a proper genetic assessment of captive-born individuals that are highly variable and exhibit low inbreeding values, we facilitate the selection of animals for reintroduction programs in order to increase the chances of restoration programs of this top predator in Europe.

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Assessment of urban climate resilience – theoretical framework and classification of indicators

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In the face of the growing impacts of climate change, cities are becoming key areas for implementing adaptation measures and building the resilience of socio-natural systems. At the same time, the concept of urban resilience remains conceptually heterogeneous, and its operationalization in empirical research relies on diverse indicators with varying scope, structure, and degree of integration of ecological and social components. However, a coherent methodological framework is lacking that would allow for the identification and analysis of these indicators.

The aim of this study is to present a methodology for analyzing urban resilience indicators used in the context of climate change and to propose a structured classification of these indicators as a tool supporting their further operationalization. The study is based on a systematic review of the scientific literature on urban resilience in the context of climate change adaptation. The results of the ongoing interdisciplinary project "Urban Resilience in the Face of Civilizational Challenges and the Sustainable Development Goals: Operationalization, Measurement, and Dissemination of the Concept" at the University of Lodz, as part of the IDUB program, which developed an integrated, cross-sectoral resilience indicator at the city level. The presented approach expands on these assumptions, proposing their deepening and refinement in relation to specific public spaces, while maintaining consistency with the previously developed framework.

The developed methodology encompasses the selection of publications, the identification and extraction of indicators, and multi-criteria analysis, taking into account definitions, scope of application, level of aggregation, spatial scale, and relationships between environmental and social components. Particular attention was paid to the conceptualization of processes related to the functioning of the water cycle, microclimate regulation, ecosystem services, and blue-green infrastructure, treating them as essential elements of systemic integration. The proposed methodological and organizational framework provides a foundation for further research on the operationalization of urban resilience, enabling more consistent design and assessment of indicators used in the planning and implementation of adaptation measures in cities.

The role of HY5 in red light-induced anthocyanin biosynthesis in *Solanum lycopersicum* L.

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Light is a key environmental regulator of plant growth, development, and photosynthesis, modulating processes including seed germination, de-etiolation, organogenesis, flowering, and seed maturation. Light-emitting diodes (LEDs) provide a controllable radiation source capable of enhancing biomass accumulation and stimulating the biosynthesis of bioactive compounds while maintaining high energy efficiency. At the molecular level, the perception of specific wavelengths by photoreceptors activates signaling networks that integrate hormonal and metabolic pathways, thereby fine-tuning plant physiological responses to environmental cues.

This study aimed to elucidate the regulatory role of the HY5 (ELONGATED HYPOCOTYL 5) transcription factor in red light-induced anthocyanin biosynthesis in tomato (*Solanum lycopersicum* L. cv. Black Cherry). Plants were cultivated under controlled environmental conditions in phytotron growth chambers with a constant photosynthetic photon flux density (PPFD) of 100 µmol m^{-2} s^{-1}. The experimental treatments included monochromatic red (100R) or blue (100B) light, as well as combined red–blue regimes (75:25, 50:50, and 25:75), with white light (RGB) serving as the control.

Monochromatic red light markedly impaired photosynthetic performance, as indicated by reductions in maximum PSII efficiency (Fv/Fm), effective quantum yield [Φ(PSII)], photochemical quenching parameters (qP, qL), and electron transport rate (ETR). Increased ΦNPQ reflected activation of photoprotective mechanisms, whereas elevated ΦNO suggested enhanced non-regulated energy dissipation associated with photodamage. Red light exposure promoted HY5 activation, which was accompanied by enhanced anthocyanin accumulation. Upregulation of HY5 was associated with increased activity of chalcone synthase (CHS), a key enzyme of the anthocyanin biosynthetic pathway, indicating transcriptional control of secondary metabolism under red light conditions. Red light further suppressed COP1-mediated ubiquitination, thereby promoting HY5 stabilization and reinforcing transcriptional activation of downstream biosynthetic genes. Collectively, these findings highlight the central role of HY5 in mediating light-quality-dependent regulation of anthocyanin biosynthesis and provide mechanistic insight into the interplay between photosynthetic performance and secondary metabolism in tomato cultivated under LED-based systems.

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Chromosomal instability induced by caffeine and ethylenediaminetetraacetic acid (EDTA) in root meristem cells of *Vicia faba*

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Chromosomal instability (CIN) in plant cells involves numerical and structural alterations to chromosomes which are caused by disorders in DNA replication and chromosome condensation and/or segregation. Disruptions to the normal organisation of chromosomes can lead to errors in segregation and the formation of acentric fragments (e.g., as a result ofdouble-strand DNA breaks), which threatens the integrity of the genome.

In plants, CIN may be associated with changes in ploidy levels, although polyploidization may have adaptive significance. Consequences of ploidy disorders include aneuploidy, both numerical and structural, and the formation of chromosomal rearrangements, which can worsen instability during subsequent cell cycle.

The experiment included the following variants: (i) a control sample; (ii) treatment with 2.5 mM hydroxyurea (HU) for 24 or 32 hours to induce replication stress; and (iii) induction of premature chromosome condensation (PCC): HU (2.5 mM for 24 hours), followed by HU and caffeine (CF; 5 mM for 8 hours). Additionally, combinations involving EDTA (a Ca^{2+} chelator used in combination with HU and HU+CF) were analysed. The morphology of interphase nuclei, the ultrastructure of mitotic chromosomes, the frequency and mechanisms of micronuclei formation and the nature and extent of chromatin condensation disorders were evaluated.

Hydroxyurea treatment resulted in significant chromatin decondensation and interphase nuclei enlargement, with heterochromatin clusters present. Inducing PCC with CF disrupted the S-M transition checkpoint and significantly increased the frequency of micronuclei generated in both mitosis-dependent and -independent manners. EDTA exacerbated symptoms of chromosomal instability, such as thickened, irregular chromosomes with numerous breaks.

When Does a Pesticide Become a Systemic Toxicant? The Case of Chlorpyrifos

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Organophosphorus pesticides, initially developed as chemical warfare agents, were later repurposed for agricultural applications following World War II. Owing to their high efficacy in pest control, they progressively replaced chlorinated insecticides and became the predominant class of plant protection chemicals worldwide. Today, organophosphate insecticides account for nearly half of all pesticides used globally, with chlorpyrifos (CP) recognized as one of the most extensively applied compounds within this group and registered in nearly 100 countries.

Beyond their classical acetylcholinesterase inhibition, CP and its bioactive metabolite, chlorpyrifos-oxon (CPO), exert broad toxic effects, including oxidative stress, inflammation, and epigenetic dysregulation. Increasing evidence links exposure to adverse neurodevelopmental, metabolic, and carcinogenic outcomes, even at low doses. These compounds interfere with key cellular signaling pathways and disrupt tissue homeostasis across multiple organ systems, highlighting their multi-target toxic potential.

Despite regulatory restrictions in several regions, chlorpyrifos remains a persistent public health concern due to its environmental stability and continued detection in food and biological samples. Ongoing exposure, particularly through imported agricultural products, underscores the need for biomonitoring and continued mechanistic research into the long-term effects of low-dose exposure.

Our *in vitro* studies conducted on murine hepatocyte AML12 cells and murine hippocampal neuronal HT22 cells demonstrated that both CP and CPO exert significant cytotoxic effects. Exposure to these compounds resulted in a marked reduction in cell viability accompanied by oxidative stress induction, as evidenced by enhanced reactive oxygen species (ROS) generation and disturbances in cellular redox homeostasis.

[1]

Transcriptomic evidence for the involvement of ApnAs and CpnCs in cadmium stress signaling in *Vitis vinifera* cells

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Dinucleoside polyphosphates (Np_nNs), including diadenosine polyphosphates (Ap_nAs) and dicytidine polyphosphates (Cp_nCs), are uncommon nucleotides considered potential signaling molecules in plants [1], although their precise physiological roles remain unclear. Cadmium (Cd) stress induces Np_nN accumulation in microorganisms [2], suggesting a possible role in heavy-metal stress responses. Previous studies have shown that Np_nNs modulate phenylpropanoid metabolism in *Arabidopsis thaliana* and *Vitis vinifera* [3, 4]. We have found that Ap_3A enhances the phenylpropanoid pathway and stilbene production in grape cell suspension cultures, indicating its involvement in cellular regulatory processes. Cd stress induces extensive transcriptional reprogramming and activates defense-related pathways, including phenylpropanoid metabolism [5]. Based on these observations, we hypothesize that Ap_nAs and Cp_nCs may contribute to transcriptional responses induced by Cd stress in grapevine cells.

Vitis vinifera cell suspension cultures were treated for 3 hours with either 5 µM Np_nN (Ap_3A, Ap_4A, Cp_3C, or Cp_4C) or 50 µM $CdCl_2$. mRNA libraries were prepared from isolated RNA, assessed using Qubit and Agilent 2100, and sequenced on NovaSeq X Plus. Raw reads were filtered, and differential gene expression analysis was performed using CLC Workbench. Comparative transcriptomic analysis was conducted to identify shared differentially expressed genes (DEGs) between Np_nN- and Cd-treated cells. Selected gene expression changes were validated by qPCR.

Transcriptomic analysis revealed extensive transcriptional responses to both Np_nN and Cd treatments. Comparative analysis identified 278 shared DEGs between Ap_3A and Cd, 34 between Ap_4A and Cd, 27 between Cp_3C and Cd, and 34 between Cp_4C and Cd treatments (FDR ≤ 0.05). Further functional analyses will enable identification of the shared metabolic and signaling pathways in which Np_nN participates during the cadmium stress response.

The identification of shared transcriptional responses between Np_nN- and Cd-treated grapevine cells indicates a relationship between Np_nN -induced and Cd-induced transcriptional regulation. The overlap in DEGs and enriched pathways suggests that similar regulatory networks may be activated in response to both treatments. These findings provide new insights into the molecular responses of grapevine cells to Np_nNs and Cd stress and support further investigation into their potential role in plant stress signaling.

Acknowledgments

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Changes in elemental composition and morphology of oat under phosphorus deficiency and struvite application

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Phosphorus (P) is an essential macronutrient required for plant growth and development, playing a key role in energy transfer, nucleic acid synthesis, membrane structure, and signal transduction [1]. Despite its importance, P availability in agricultural systems is often limited by its low solubility and strong fixation in soil, which significantly reduces plant uptake efficiency. Consequently, P deficiency remains a significant constraint on crop productivity worldwide. Conventional phosphorus fertilisers are primarily derived from non-renewable phosphate rock resources, whose reserves are being progressively depleted [2]. Struvite is a recycled phosphorus compound recovered from wastewater streams and is increasingly recognised as a slow-release fertiliser. Several studies have demonstrated that struvite can effectively supply phosphorus to crops. However, plant responses depend on struvite formulation, particle size, and cultivation conditions [3]. Oat (*Avena sativa*) cv. Motto (registered in 2023) was cultivated hydroponically under controlled conditions using liquid nutrient solutions differing in P availability and source. The experimental treatments included: adequate P supply (+P, KH_2PO_4), P deficiency (-P, no phosphorus), and three struvite-based P sources: Struvite Crystal Green Pearl (SP), Struvite Crystal Green (ST), and Struvite Crystal Green Synchro 50 (S50). After three weeks of cultivation, the elemental composition of oat leaves was determined using inductively coupled plasma mass spectrometry (ICP-MS), with particular emphasis on phosphorus, manganese, iron, potassium, calcium, copper, zinc, and cadmium. In addition, P content in dried leaf tissues was examined by energy-dispersive X-ray spectroscopy (EDX). Leaf morphology was assessed using both stereomicroscopy (binocular microscopy) and scanning electron microscopy (SEM), while total phenolic content was measured as an indicator of plant stress response. The results demonstrated that phosphorus deficiency altered elemental composition, leaf morphology, and phenolic content in oat plants. Application of struvite-based fertilisers mitigated several adverse effects of P limitation, although the extent of the response depended on the struvite formulation used. These findings indicate that struvite can serve as an effective alternative phosphorus source in oat cultivation.

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Development of a new analytical method for the determination of ß-blockers and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) residues using LC-(ESI)-MS/MS in environmental samples

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The monitoring of pharmaceutical residues in wastewaters and river water is essential for safeguarding water quality. Pharmaceuticals are released into the aquatic environment primarily through municipal wastewater, as a consequence of human excretion, improper disposal of unused medicines, and discharges from healthcare facilities and pharmaceutical manufacturing [1]. Growing regulatory attention, including the recently revised EU Urban Wastewater Treatment Directive (UE) 2024/3019, underscores the need for enhanced monitoring and more effective removal of micropollutants, among which pharmaceuticals represent a priority group. Over the past decade, these compounds have therefore become a major focus of environmental research, driven by the global rise in drug consumption, demographic shifts, and continuous advances in pharmaceutical development. Although typically detected at low concentrations, pharmaceuticals are specifically designed to exert biological activity at very low doses, raising concerns about their potential environmental impact even at trace levels [2].

β-blockers and non-steroidal anti-inflammatory drugs (NSAIDs) are among the pharmaceuticals most frequently detected in municipal wastewater and surface waters. Their widespread use, chemical stability, and the limited removal efficiency of conventional wastewater treatment processes contribute to their continuous release into the aquatic environment. Despite occurring at trace concentrations (ng/L to µg/L), these compounds may still pose risks to aquatic organisms and ecosystems. Importantly, most environmental studies focus primarily on parent compounds, whereas the determination of their metabolites is often overlooked. This may lead to an underestimation of the actual environmental burden, as certain metabolites can exhibit comparable or even higher ecotoxicity than their parent drugs and may differ in terms of persistence and mobility [3].

In the present study, an LC–MS/MS method was optimized for the simultaneous determination of 12 βblockers, 7 NSAIDs, and selected metabolites in influent and effluent wastewater, as well as in river water. Environmental samples were preconcentrated by solid-phase extraction using Strata-X cartridges (Phenomenex, 200 mg/3 mL). Preliminary findings confirmed the presence of the target pharmaceuticals in both wastewater and river water samples.

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CRY1-Dependent Spectral Signal Integration Controls Photomorphogenesis and Metabolic Plasticity in Tomato Seedlings

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Light-driven developmental reprogramming in plants is orchestrated by photoreceptors that integrate spectral cues into coordinated morphogenetic and metabolic outputs. Here, we investigated the role of cryptochrome 1 (CRY1) in regulating photomorphogenesis, photosynthetic performance and secondary metabolism in tomato (*Solanum lycopersicum* L., cv. Blackball) seedlings exposed to monochromatic red (630 nm), green (515 nm), blue (455 nm), blue supplemented with UV-A (444 + 399 nm), and mixed RGB spectrum. Seedlings with functional CRY1 were compared with plants subjected to chemical inhibition of CRY1 using 3-bromo-7-nitroindazole (3B7N).

Red and green light promoted hypocotyl elongation, whereas blue light markedly suppressed axial growth, consistent with the dominant inhibitory role of blue-light signaling in seedling morphogenesis. The B+UV treatment enhanced root elongation and induced the highest accumulation of chlorophyll *a*, chlorophyll *b* and carotenoids, indicating a synergistic interaction between B and UV-A radiation. CRY1 inhibition substantially altered both morphological and biochemical responses, enhancing elongation under G light and reducing photosynthetic pigment content. Our analyses also revealed that the highest accumulation of phenolic compounds in the examined seedlings occurred under monochromatic B light in the absence of CRY inhibition. In contrast, phenolic content was markedly reduced under monochromatic R or G light.

Chlorophyll *a* fluorescence analysis revealed the highest effective quantum yield of photosystem II [Y(II)] under RGB and B/B+UV, whereas monochromatic R light resulted in the lowest photosynthetic efficiency. Elevated non-photochemical quenching [Y(NPQ)] under R and G light indicates activation of photoprotective mechanisms. Notably, in the B+UV treatment combined with CRY1 inhibition, increased accumulation of key photosynthetic proteins (Rubisco large and small subunits, light-harvesting complex proteins, ATP synthase) was observed, suggesting compensatory reorganization of the photosynthetic apparatus under attenuated cryptochrome signaling.

Collectively, our findings identify CRY1-dependent signaling as a central regulatory node integrating spectral information to modulate early developmental programs and metabolic plasticity in tomato seedlings. Notably, the analyses raise an especially intriguing possibility that CRY1 contributes to signal integration beyond its canonical blue-light absorption range, potentially extending into the green and UV light spectrum.

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Aromatic plant use in blue tit nests across Europe – preliminary findings

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Aromatic plant use in avian nests has long been reported in several scattered local studies, but its geographic prevalence and ecological relevance across broader spatial scales remain poorly understood. Here, we provide the first continent-wide assessment of the occurrence of aromatic plants in blue tit (*Cyanistes caeruleus*) nests across Europe.

During the 2024 breeding season, eleven research teams collected standardized data from nine European countries and 16 study areas, documenting the presence and identity of plant material incorporated into nests. Across all populations, blue tits used 34 species of aromatic plants as nest additions.

The proportion of nests containing such plants varied among populations, ranging from 0% in the Czech Republic to 100% in one Corsican population. This large-scale comparative study demonstrates that the use of aromatic plants during the breeding season is widespread but highly variable across Europe, revealing substantial spatial heterogeneity in this behaviour. Our findings provide the first comprehensive evidence for the continent-wide occurrence of aromatic plant use in blue tit nests and establish a foundation for future work on its ecological drivers.

Impact of fungal biodegradation on the ecotoxicity of selected drugs in a microplate algal bioassay

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Long-term pharmacotherapy of chronic diseases contributes to an increase in global drug consumption. Furthermore, inadequate elimination of drug residues during wastewater treatment results in their accumulation in the environment and associated ecotoxicological effects. Microbial degradation offers a promising and environmentally sustainable strategy for the removal of pharmaceutical contaminants. The presented study aimed to evaluate fungi-mediated biotransformation efficiency of selected pharmaceuticals and to assess the impact of mycological biodegradation on their ecotoxicity.

Three strains of *Cunninghamella* spp. fungi (*C. echinulata*, *C. blakesleeana*, and *C. elegans*), as well as eight drugs (diclofenac, ibuprofen, indomethacin, naproxen, carbamazepine, amitriptyline, fluoxetine, and donepezil) were used in the study. Mycological biotransformations were conducted for 7 days and analyzed via UHPLC–MS. The ecotoxicity of samples was assessed using a modified microplate bioassay utilizing green alga *Pseudokirchneriella subcapitata*[1,2].

Biotransformation efficiency varied significantly among *Cunninghamella* strains. Ibuprofen was completely metabolized by all strains, while high biodegradation rates were also observed for diclofenac (91%, *C. elegans*), indomethacin (90%, *C. blakesleeana*), naproxen (92%, *C. elegans*), amitriptyline (100%, *C. echinulata*), and donepezil (94%, *C. elegans*). In contrast, carbamazepine and fluoxetine showed limited biodegradation, reaching maxima of 30% and 34%, respectively. Ecotoxicity assay revealed reduced toxicity of *Cunninghamella*-treated samples compared to parent compounds for diclofenac, ibuprofen, indomethacin, naproxen, carbamazepine, and amitriptyline, whereas increased ecotoxicity was observed for transformation products of fluoxetine and donepezil.

The results demonstrate the ability of *Cunninghamella* strains to biodegrade tested pharmaceuticals, while the effects of fungal biotransformation on ecotoxicity were compound-dependent.

Acknowledgments

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Life in the Dark: Characteristics of a Subterranean Population of the Stone Crayfish

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The stone crayfish, Austropotamobius torrentium (Schrank, 1803), is the smallest of the seven native European crayfish species. It is protected by European and Croatian nature conservation directives and laws. Its distribution in Croatia within the Northern Central Dinarides presents a biodiversity hotspot for the species [1]. Conservation of genetically diverse populations is considered one of the cornerstones of modern conservation biology [2]. Cave Sušik in Croatia hosts a rare, geographically isolated subterranean population of the stone crayfish. Caves can serve as model habitats for studying evolutionary adaptations of species to new environments and, in the context of climate change, can act as potential refugia. [3]. Within the scope of this study, we determined the population and genetic characteristics of the Sušik population of the stone crayfish and established the baseline for future studies.

This study took place between July and October of 2023. In total, we weighed, measured, and sexed 181 individuals. Measured population characteristics indicate a stable population, with 1.01 individuals per m^2 and an even sex and size-class distribution. We also note deviations from the usual year cycle (e.g., the earlier presence of attached spermatophores in late August and the presence of moulting individuals only in July). These deviations may indicate an early stage of adaptation to the subterranean environment. To assess population genetic diversity, we performed microsatellite genotyping and sequenced the mitochondrial *COI* gene. DNA was extracted from the pereiopodal muscle tissue of 30 individuals. Phylogenetic analysis placed all individuals within the established evolutionary significant unit and phylogroup Žumberak, Plitvice, and Bjelolasica, comprising two known haplotypes. Haplotype analysis also suggests the presence of two unique haplotypes differing by one mutational step from the known ones. Microsatellite analysis revealed poor gene flow between the studied population and geographically close populations within the same phylogroup. We observed a high number of private alleles, which can indicate a sampling bias of a small cave population, but also suggest the studied population's isolation. This study, which reports the first cave population of stone crayfish in Croatia, provides a baseline for future studies of subterranean adaptations in this and related species.

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Connecting the Dots: Combining Remote Sensing and eDNA to Characterise Temporary Ponds.

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Temporary ponds are unique lentic ecosystems characterized by a recurrent cycle of flooded and dry phases. These habitats function as critical biodiversity hotspots and evolutionary refugia for highly specialised organisms. However, because they are dry for much of the year and occupy small surface areas, they remain "cryptic" and undocumented in official land-cover inventories. Furthermore, their hydroperiods are becoming increasingly unpredictable due to the shifting precipitation patterns of a changing climate, threatening the survival of species that are dependent on specific dormant phases. The study was implemented in Apulia across a latitudinal and bioclimatic gradient encompassing the Gargano, Murgia, and Salento regions. Using 15 temporary ponds (n=5 per region) as a structural baseline, we performed geomorphometric characterization based on high-resolution Digital Surface Models and Digital Terrain Models. By implementing automated sink-extraction and isolating closed topographic depressions, the model was deployed to scan the landscape surrounding the reference sites systematically. This enabled the detection of previously unmapped depressional landforms whose geometric containment and volumetric properties indicate the physical capacity for seasonal water ponding. To evaluate the molecular landscape and taxonomic distribution of these habitats, we performed environmental DNA (eDNA) metabarcoding. Water samples were collected from each pond and processed via on-site filtration, pulling the water through specialised filters (Sylphium) to capture environmental DNA. DNA was extracted from the filters and the cytochrome c oxidase subunit 1 (CO1) mitochondrial region was amplified. This molecular approach provided high-resolution taxonomic inventories of specialized invertebrate communities (e.g., Arthropoda, Rotifera and Annelida). We performed a comparative analysis of these assemblages to quantify intra-regional similarity versus inter-regional turnover, assessing how geographical distance and physical basin architecture influence community composition and molecular diversity across the Apulian peninsula freshwaters. Preliminary results indicate that the geomorphometric model significantly increases detection sensitivity in complex terrains compared to existing regional land-cover datasets. By pairing terrain-based predictive modeling with CO1-derived biological validation, this study establishes a scalable, non-invasive protocol for the monitoring of temporary ponds. This integrated framework provides a robust empirical foundation for quantifying regional connectivity and prioritizing conservation interventions for endangered and transient Mediterranean biodiversity, ensuring the protection of vulnerable invertebrate assemblages within overlooked habitats.

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Rhizomes as a Pathway for Mycobiome Transfer in Clonal Plant Networks

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Clonal plants form underground networks of ramets connected by rhizomes, which may facilitate not only the transport of water and assimilates but also the movement of microorganisms. The aim of this study was to determine whether endophytic fungi are transferred between neighboring ramets within naturally functioning clonal networks of *Maianthemum bifolium*, and whether rhizomes serve as a reservoir and potential pathway for the dispersal of the mycobiome.

The study was conducted on 30 fragments of clonal networks (89 ramets) from three sites located in Scots pine forests of the Śrem Forest District (Wielkopolska, Poland). Fungi were isolated in vitro from leaves and rhizome internodes following surface sterilization. A total of 256 pure isolates were obtained and molecularly identified based on ITS rDNA sequences (≥98% similarity).

In total, 52 fungal taxa representing 23 genera were recorded, predominantly belonging to Ascomycota and Basidiomycota. Some taxa were specific to either leaves or rhizome internodes, whereas selected taxa occurred in both tissue types as well as in neighboring ramets. The presence of identical taxa in connecting structures and ramets suggests the possibility of fungal movement within the clonal network.

The results indicate that rhizomes of *M. bifolium* may function as a pathway for the horizontal transfer of endophytes under natural conditions, although this transfer appears to be significantly less pronounced than that observed under experimental conditions. The variability in mycobiome composition also highlights the influence of local environmental factors and alternative sources of colonization, thereby extending the concept of clonal integration to a microbiological dimension. These findings suggest that the functioning of clonal plants in ecosystems should be considered in the context of the metaholobiont.

ABSTRACTS:

MOLECULAR AND MEDICAL BIOLOGY

Ketogenic diet induces metabolic and myogenic remodeling of skeletal muscle in a DMM-induced osteoarthritis model

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Osteoarthritis (OA) is a prevalent degenerative joint disease. One of the risk factors for OA is obesity, due to increased mechanical load to the joints. In patients with OA, weight reduction is recommended to alleviate symptoms such as pain and inflammation. Recently, the ketogenic diet (KD) has gained attention as a potential therapeutic dietary strategy in OA, however most of the currently ongoing studies do not explore the impact of KD on skeletal muscle function in OA. In case of OA, maintenance of muscle mass and proper muscle function is important, since skeletal muscle wasting can weaken joint support, impair movement and compromise stability.

In our study, we investigated whether a metabolic shift toward enhanced fatty acid oxidation (FAO) and ketone body utilization could support skeletal muscle remodeling and preserve muscle mass under conditions of metabolic stress and joint destabilization. We designed experimental *in vivo* model, where C57BL/6J mice were first exposed to an obesogenic high-fat diet and subjected to destabilization of the medial meniscus (DMM) surgery to induce OA. Following surgery, animals were maintained on high-fat diet, or switched to either standard diet, or ketogenic diet. Later, gastrocnemius muscles were collected for molecular analyses using qPCR and Western blotting.

We observed that KD-fed mice exhibited reduced glycemia and elevated circulating β-hydroxybutyrate levels, confirming sustained ketosis. In skeletal muscle, KD induced upregulation of FAO-related genes, including Cpt1b and Pdk4, which together with modulation of adiponectin signaling, indicates metabolic reprogramming toward lipid utilization. Notably, KD was associated with increased expression of early myogenic markers, including Pax7 and MyoD1, suggesting altered satellite cell activation. Despite significant body weight reduction, absolute muscle mass was preserved and no reduction in score for KD-fed animals was observed during functional assessment using the Kondziela test.

Collectively, our findings demonstrate that, in a murine model of obesity compounded OA, KD induces metabolic remodeling in skeletal muscle, accompanied by modulation of satellite cell-related markers. Our results indicate that KD promotes adaptive muscle remodeling in a metabolically stressed OA model.

Acknowledgments

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Glial-to-Mesenchymal Transition of Müller Cells in Proliferative Vitreoretinopathy: A Preliminary *In Vitro* Study

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Background: Proliferative vitreoretinopathy (PVR) remains the leading cause of failure after surgery for rhegmatogenous retinal detachment. Although epithelial-to-mesenchymal transition (EMT) of retinal pigment epithelial cells is considered a central mechanism in PVR pathogenesis, the contribution of Müller glial (MG) cells remains insufficiently explored. Emerging evidence suggests that MG cells may undergo glial-to-mesenchymal transition (GMT), a process analogous to EMT, potentially contributing to fibrotic membrane formation.

Purpose: To investigate the role of Müller cell glial-to-mesenchymal transition in the etiopathogenesis of PVR using an *in vitro* model stimulated with vitreous fluid derived from PVR patients.

Methods: The human Müller cell line MIO-M1 was exposed to pooled vitreous fluid collected from patients undergoing vitrectomy for PVR or transforming growth factor-β (TGF-β) in order to induce GMT in the control group. After 24-hour stimulation, cellular proliferation was assessed using XTT assay, migratory capacity was evaluated by wound healing assay, and phenotypic changes were analyzed by qRT-PCR. Expression of glial markers (GLUL, SOX9), mesenchymal markers (ACTA2/α-SMA, TAGLN), the transcription factor SNAI1, and extracellular matrix-related genes (COL1A1, FN1) was measured, and morphological changes of Müller cells were evaluated after treatments.

Results: Preliminary observations suggest that exposure to PVR-derived vitreous fluid promotes increased proliferative and migratory activity of Müller cells compared to controls. Molecular analyses are expected to demonstrate downregulation of glial markers accompanied by upregulation of mesenchymal and extracellular matrix-associated genes, consistent with induction of glial-to-mesenchymal transition. Morphological assessment revealed phenotypic changes characteristic of mesenchymal-like transformation. These findings indicate that soluble factors present in PVR vitreous may contribute to phenotypic reprogramming of Müller cells.

Conclusions: Our findings support the hypothesis that glial-to-mesenchymal transition of Müller cells may represent an underrecognized mechanism in PVR pathogenesis. Targeting cytokine-driven Müller cell plasticity could provide novel therapeutic strategies aimed at reducing fibrotic membrane formation and surgical failure rates.

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Prognostic Significance of YY1AP1 in Colorectal Cancer

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Introduction: Colorectal cancer (CRC) remains one of the leading causes of cancer-related mortality worldwide. Increasing evidence suggests that proteins involved in transcriptional regulation and chromatin remodeling may play a significant role in CRC progression. YY1AP1 (YY1-associated protein 1) is a transcriptional co-regulator implicated in gene expression control; however, its role in CRC has not yet been clearly defined.

Aim: The aim of this study was to evaluate YY1AP1 expression levels in colorectal cancer tissues and adjacent normal tissues, and to analyze their association with clinicopathological features and overall survival of patients.

Materials and Methods: The study group consisted of 118 patients diagnosed with colorectal cancer. For 42 cases, histologically normal tissues adjacent to the tumor were available and served as controls. Immunohistochemical staining was performed to assess YY1AP1 expression in CRC and matched normal tissues. Expression levels were evaluated semi-quantitatively using the H-score method and correlated with clinicopathological data and overall survival.

Results: YY1AP1 expression was detected in both the nucleus and cytoplasm of cancer cells. YY1AP1 expression was significantly higher in tumor tissue compared to adjacent normotypic tissue. Moreover, higher YY1AP1 levels correlated with advanced disease stage and poorer prognosis.

Conclusions: YY1AP1 is overexpressed in colorectal cancer compared to adjacent normal tissue, and its nuclear and cytoplasmic expression is associated with more advanced disease. These findings suggest that YY1AP1 may serve as a potential marker of CRC progression and justify further studies to elucidate its prognostic and functional significance.

Effects of surgically induced estrogen deprivation on intestinal function and morphology in mature and immature NRF2 knockout female mice.

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The gastrointestinal system relies on intricate interactions between multiple cell types and signaling pathways. NRF2 plays a key role in maintaining gut function by regulating cellular stress responses and preserving intestinal homeostasis. In our previous studies we showed that absence of NRF2 transcriptional activity (NRF2 tKO) leads to significant microscopic changes in the colon without affecting general mouse physiology and that oestrogens may play a significant role in this process. To further evaluate the topic, we aimed to verify if NRF2 transcriptional inactivity (TA) alters intestinal function and morphology in pre- mature and mature female mice subjected by ovariectomy.

In the study we used 4- and 12-week-old NRF2 tKO female mice who underwent ovariectomy or sham operation. Mice were subjected to functional tests of intestinal function such as whole gastrointestinal transit (WGT), colon bead expulsion and defecation before and two weeks after operation. Finally, histological samples of intestines were collected for further analysis.

The results showed that premature ovariectomized mice have prolonged WGT ($p<0.05$), thinner intestinal wall ($p<0.05$), abut higher large and small intestine weight/body weight index ($p<0.05$) compared to mature ovariectomized mice, indicating a strong impairment of global gastrointestinal transit in younger mice. In contrast, the colon bead assay revealed longer expulsion time ($p<0.05$) in mature ovariectomized mice, suggesting dysfunction in distal colonic motility. We also noticed alterations in histology associated with higher inflammatory cells influx to lamina propria of the colon samples in mature OVA NRF2 tKO mice compared to sham mice.

In conclusion, lack of NRF2 transcriptional activity leads to changes in intestinal function and structures in mature rather than pre-mature mice. This may suggest an interplay between NRF2 transcriptional activity and estrogen influence on intestine function.

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Homocysteine and homocysteine thiolactone contribute to Alzheimer's disease *via* TAU modifications in N2A-APPswe cells

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Background: Elevated levels of homocysteine (Hcy) and/or homocysteine thiolactone (HTL), are linked to neurodegenerative diseases, including Alzheimer's disease (AD). Hyperphosphorylation or elevated acetylation of TAU protein are hallmarks of AD. However, the mechanistic roles of Hcy and HTL in the development and progression of AD are not fully understood.

Aim: We tested a hypothesis that Hcy and HTL promote TAU accumulation via hyperphosphorylation and acetylation of TAU in mouse neuroblastoma N2A-APPswe cells.

Methods: Neuroblastoma N2A-APPswe cells (N2A-APPswe) harboring a human transgene with mutation in the amyloid precursor protein (APP) gene were grown on the complete DMEM/F12 medium. Cells were treated with 20-200 µM Hcy or HTL. Phosphorylation and acetylation of TAU, as well as selected enzymes involved in TAU modification, were quantified by Immunofluorescence and Western blotting.

Results: Immunofluorescent analysis showed that Hcy and HTL upregulate phosphorylated TAU at threonine 205 and serine 396, and acetylated TAU at lysine 174, relative to untreated cells. However levels of total TAU were not affected in cells after Hcy and HTL treatment compared to control cells. At the same time Western blots showed upregulation of CDK5 and GSK3α after Hcy and HTL treatments compared to untreated cells, while GSK3β level was not affected.

Conclusion: Treatment with Hcy or HTL affects TAU modifications which in turn promote TAU aggregation in mouse neuroblastoma N2A-APPswe cells. The hyperphosphorylated TAU at Ser396 and at Thr205 results from elevated levels of TAU kinases - CDK5 and GSK3α, which are upregulated by Hcy and HTL treatments.

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Impact of acrolein and glyoxal on membrane fluidity and antioxidant defense systems in human peripheral blood mononuclear cells

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Acrolein (ACR) and glyoxal (GO) are highly reactive aldehydes. They can be generated endogenously through various processes, such as lipid peroxidation, glycation reactions, and inflammation. Both toxins can also come from environmental sources, including cigarette smoke, combustion products, and highly processed food. The rapid absorption of these toxins leads to a significant increase in their concentration in the bloodstream. Elevated levels of ACR and GO in the human body are associated with the pathogenesis of various diseases, including atherosclerosis, diabetic complications, neurodegenerative disorders, and kidney diseases.

These carbonyl compounds promote oxidative stress by modifying essential biomolecules and forming protein and nucleic acid adducts, a process known as carbonyl stress. They also cause the depletion of intracellular glutathione (GSH). Regarding amino acids, the toxins primarily bind to thiol, amino, or imidazole groups. Such protein modifications trigger a cascade resulting in organelle damage, cellular dysfunction, and cell death. Additionally, both aldehydes stimulate the production of reactive oxygen species (ROS), which disrupts the redox balance and promotes oxidative stress.

Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats and subjected to treatment with ACR (30, 60, and 90 µM) or GO (2, 5, and 10 mM) for 24 hours at 37 °C. After incubation, the oxidative changes in the cells were evaluated.

Cell membrane fluidity was evaluated by means of TMA-DPH and DPH fluorescent labeling. A FRAP assay was employed to evaluate the total non-enzymatic antioxidant capacity of the cells. Glutathione peroxidase (GPx) activity in the PBMC was determined following the assay kit (Catalog Number: MAK437). Superoxide dismutase (SOD) and catalase (CAT) expression in the PBMC was determined by Western blotting. Statistical analysis of the obtained results was performed using Statistica v. 13.3.

The results of the study demonstrated that treatment of PBMCs with ACR and GO leads to a significant increase in cell membrane fluidity at the depth of the 5th carbon atom of the fatty acid chain. In turn, at a depth of the 12th carbon atom, a slight stiffening of the cell membrane was observed. The increased surface fluidity likely results from lipid peroxidation and fatty acid fragmentation. Conversely, aldehyde-induced cross-linking and lipid-protein aggregates likely cause rigidification of the hydrophobic core. Both toxins induced a significant decrease in non-enzymatic antioxidant potential and a reduction in GPx activity. The decrease in GPx activity may result from the binding of toxins to cysteine residues within the enzyme's active site. This leads to a collapse of the GSH/GSSG ratio and a subsequent reduction in the non-enzymatic antioxidant potential. Furthermore, changes in the expression of key defense enzymes, SOD and CAT, were observed in cells exposed to ACR and GO. The results suggest that ACR and GO cause damage to the transcriptional machinery or mRNA degradation occurs. Additionally, these aldehydes may modify histones, thereby blocking the gene expression of SOD and CAT.

The findings suggest that environmental and endogenous exposure to ACR and GO may contribute to systemic inflammatory and degenerative processes by oxidative damage in PBMC cells.

Arginine-rich DOPE/TAP liposomes and their interaction with cell membranes and proteins

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The most common proposed method for administering nanoparticles is intravenous injection. This carries certain risks, therefore, an important aspect of characterizing the properties of nanoparticles is understanding their interaction with cell membranes and blood serum proteins. Does the additional presence of siRNA influence the interaction of liposomes?

We characterized DOPE/TAP/R9-chol liposomes by determining their effect on membrane fluidity by measuring the fluorescence aniotropy of TMA and TMA-DPH. We then determined the fluorescence quenching of transferrin and human albumin. Finally, we determined the changes in circular dichroism of these proteins. In order to understand the formation of the protein corona, we measured the hydrodynamic diameter of the formed agglomerates. Additionally, we took TEM images to illustrate the interaction of liposomes and their complexes with siRNA.

The results confirm the absence of changes in membrane fluidity under the influence of DOPE/TAP/R9-chol liposomes. However, we observe protein binding by the liposome, which is diminished by the presence of siRNA.

Hyperglycemia modulates expression of *GLUT1* and *RRAD* genes in differentiating adipocytes

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Background:

Visceral adipocytes (vAds) play an important role in maintaining glucose homeostasis, and their dysfunction contributes to glucose metabolism disorders. Their basal glucose uptake is, to some extent, exerted by insulin-independent glucose transporter (GLUT1), which can be downregulated by Ras-related GTPase (RRAD). Thus, the modulation of glucose uptake by the RRAD–GLUT1 axis in vAds may contribute to impaired glucose metabolism. However, the role of RRAD in regulating GLUT1 expression during adipogenesis and under hyperglycemic conditions remains unclear.

Aim:

The aim of this study was to evaluate the expression of *GLUT1* and *RRAD* in adipocytes differentiating under normoglycemic and hyperglycemic conditions, and to explore the potential regulatory mechanisms of this axis in the context of glucose metabolism disorders.

Methods:

Human visceral preadipocytes were differentiated into mature adipocytes over 18 days using manufacturer's media and protocol (Innoprot). Both preadipocytes and mature adipocytes were maintained under either normoglycemia or hyperglycemia (30 mM). The expression of *GLUT1* and *RRAD* was assessed using RT-qPCR and normalized to *RPLP0*. Statistical analysis was performed using t-Student test.

Results:

In hyperglycemic conditions, increased expression of both *GLUT1* and *RRAD* was observed in mature vAds. During adipogenesis, *GLUT1* mRNA levels decreased under normoglycemic conditions, whereas hyperglycemia was associated with increased *GLUT1* expression. The expression of *RRAD* increased during adipocyte differentiation, with higher expression observed under hyperglycemic conditions. Under normoglycemic conditions, changes in *RRAD* expression reflected *GLUT1* expression patterns, whereas this relationship was altered in hyperglycemia, suggesting dysregulation of mechanisms controlling insulin-independent glucose uptake.

Conclusions:

Our findings suggest that RRAD may act as a potential regulator of GLUT1 expression in vAds under physiological conditions, contributing to the control of insulin-independent glucose uptake. Disruption of this relationship under hyperglycemic conditions may reflect a compensatory or dysregulated mechanism and could contribute to impaired glucose homeostasis, highlighting the potential relevance of the RRAD–GLUT1 axis in glucose metabolism disorders.

Importance of *CCL4* in the molecular mechanism of depressive disorders

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Depression is a psychiatric disorder whose incidence has increased substantially in recent years. It currently affects approximately 332 million people worldwide and represents a major public health concern, significantly impacting all aspects of life. Clinically, it is characterized by impaired concentration, fatigue, and reduced energy level. Although pharmacological treatments have been developed, a decline in their effectiveness has been observed in some patients. Selective serotonin reuptake inhibitors (SSRIs) such as Escitalopram, are commonly prescribe; however, the prevalence of treatment-resistant depression is increasing.

The aetiology of depression is highly complex and involves the interplay of biological, social, and psychological factors. The rising number of diagnosed cases has made depression the focus of extensive research. The rising number of diagnosed cases has made depression the focus of extensive research. Nevertheless, its pathomechanism remains incompletely understood . To date, numerous theories have been proposed, highlighting the involvement of various biological pathways in its development. Increasing evidence also suggests that neuroinflammation may contribute to the pathophysiology of depression.

Therefore, this study aims to determine whether chronic mild stress (CMS) and treatment with escitalopram influence *CCL4* gene expression. The findings may help to elucidate the potential role of this gene in the molecular mechanisms underlying the development of depressive disorders.

The genetic material used in this study consisted of RNA isolated from male Wistar Han rats. Total RNA was extracted from four brain regions: the ventral hippocampus, dorsal hippocampus, the prefrontal cortex, and additional brain regions. The animals were randomly assigned to four experimental groups: control, stressed (subjected to the CMS procedure), control treated with escitalopram and stressed (subjected to the CMS procedure) treated this drug.

CCR5 mRNA expression levels were quantified using real-time polymerase chain reaction with TaqMan probes (real-time PCR). ComplementaryDNA (cDNA) was synthesized viareverse transcription prior to amplification. *PPIA* and *RPL32* were used as the reference genes for normalization of gene expression. The study protocol was approved by the Bioethics Committee of the Institute of Pharmacology of the Polish Academy of Sciences in Krakow (No. 85/2022).

Statistically significant differences were observed exclusively in the additional brain regions. However, an upward trend in gene expression was also noted in the remaining regions, further supporting the potential involvement of the *CCL4* gene in the pathogenesis of depression.

Keywords: depression, neurobiology, mRNA expression, chemokines,

The role of selected genes in the transition to a polyaneuploid state in doxorubicin-resistant triple-negative breast cancer cells

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Introduction

Polyaneuploid giant cancer cells (PGCCs) are increasingly recognized as key contributors to tumor heterogeneity and chemotherapy resistance. In triple-negative breast cancer (TNBC), genotoxic stress induced by doxorubicin promotes the emergence of multinucleated, polyploid cells with enhanced adaptive potential. Our research focused on the molecular characteristics of polyaneuploid giant cancer cells (PGCCs) arising in the MDA-MB-231 cell line, and on identifying the genes involved in this transition.

Methods

Doxorubicin-resistant and non-resistant MDA-MB-231 cells were analyzed following drug treatment or under control conditions. Single-cell RNA sequencing (scRNA-seq) was employed to assess transcriptional heterogeneity within cell populations. The data were analyzed using the usegalaxy.org platform, and functional annotation of genes was performed using Gene Ontology (GO) analysis. Genes exhibiting high expression variability were silenced using siRNA, and the impact of gene knockdown on PGCC formation and cell survival following doxorubicin exposure was evaluated.

Results

Non-resistant cells exhibited increased transcription of genes associated with programmed cell death, cell division inhibition, mitochondrial membrane depolarisation and decreased mitochondrial respiration. Two subpopulations were visible in resistant cells, which, based on GO analysis results, can be treated as early/precursor and mature PGCC cells. Cells in the early stages of polyaneuploidy were characterised by increased mitochondrial respiration and high levels of enzymes involved in removing reactive oxygen species, particularly peroxides. Unlike doxorubicin-treated basal cells, PGCC precursors exhibited reduced susceptibility to apoptosis. Further upregulation of genes responsible for oxidative phosphorylation, ribosome activity and the response to DNA damage was observed in PGCC cells. Silencing of selected gens in combination with doxorubicin treatment reduced the number of polyaneuploid cells and significantly decreased cell survival compared to doxorubicin treatment alone.

Conclusions

Transition to a polyaneuploid state represents an adaptive survival mechanism of TNBC cells under genotoxic stress. PGCCs exhibit distinct metabolic and DNA repair signatures that may contribute to chemotherapy resistance. TOPBP1 appears to play a central role in this process and may constitute a promising therapeutic target to limit polyaneuploidization and overcome doxorubicin resistance in TNBC.

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PLXNA1 and PLXNA3 Underexpressed in Depression – a preliminary study

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Neuroplasticity - brain's ability to alter both its structure and function - is vital for CNS (central nervous system) homeostasis. Its impairment is considered a hallmark of major depressive disorder (MDD). The present study aims to provide a deeper insight into this phenomenon and evaluate the expression of neuroplasticity-related genes in peripheral blood samples from patients suffering from MDD. Results were compared against a control group of healthy individuals who were not prescribed antidepressant medication and had no history of psychiatric disorders. Studied genes, chosen based on their known involvement in neuroplasticity, were PLXNA1 (Plexin A1), PLXNA3 (Plexin A3) and PLXNB2 (Plexin B2). Plexins encode proteins that act as receptors for Semaphorins – a family of axonal growth cone guidance molecules. Because of that, they participate in neural development and directing of axon pathfinding. In current research, PLXNA1 and PLXNA3 were expressed significantly lower in depressed patients compared to healthy controls. However, there was no correlation between severity of depression (Hamilton scale), age, sex, BMI (body mass index) and expression of both genes. There were no significant changes in expression of PLXNB2. Class A Plexins, such as PLXNA1 and PLXNA3 do not have additional extracellular cleavage site present in class B Plexins, for instance PLXNB2. Therefore, their expression may be regulated differently. The findings demonstrate that significant alterations in the expression of neuroplasticity-related genes occur not only within the CNS but are also detectable in peripheral tissues, such as blood.

Bisphenol S, the 'safer' bisphenol A analogue, promotes the proliferation of endometrial cancer cells

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Introduction

Hormone-dependent cancers are the most common among the human population. They are associated with physiological changes in the hormonal balance due to age, as well as environmental factors. Endocrine-disrupting chemicals (EDCs) act by interacting with estrogen receptors, thereby disrupting hormonal regulation. Bisphenol A (BPA) is a toxic compound, previously used in the chemical industry. Nevertheless, BPA has been banned due to its EDC effect in humans; however, its analogues are still used in the chemical industry. Bisphenol S (BPS) is one such analogue, and due to its structural similarity to BPA, it is highly possible that BPS also exhibits estrogenic activity. Studies have already demonstrated that BPS can promote cell proliferation and progression in breast and prostate cancer cells. However, the role of BPS in endometrial cancer (EC) remains underexplored.

Aim of the study

The study aimed to verify whether BPS induces the proliferation of EC cells.

Materials and methods

A hormone-sensitive Ishikawa cell line was used as an *in vitro* model of EC. Estradiol (E2) was used as a positive control (10 nM). The proliferation of cells was measured with the CFSE and BrdU assay after 72 h. The clonogenicity was tested with a colony formation assay after 14 days of exposure to BPS. To calculate statistical significance, One-way ANOVA was used. *p* value lower than 0.05 was considered statistically significant (GraphPad Prism software).

Results

The results showed that BPS significantly increased the proliferation of the Ishikawa cell line in all tested doses (0.01- 1 μM). Moreover, the exposure of EC cells to BPS increased the clonogenicity of the cells.

Conclusion

The results indicate that BPS, like BPA, can induce EC cell proliferation, suggesting that exposure to BPS may affect these cells and potentially contribute to carcinogenesis.

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Altered microRNAs expression associated with chronic hyperglycaemia in pancreatic cancer

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Pancreatic cancer remains a highly aggressive malignancy with poor clinical outcomes, largely due to late diagnosis and limited treatment regimens. Increasing evidence suggests that metabolic disorders, including diabetes, may contribute to pancreatic carcinogenesis.

Chronic hyperglycaemia is known to induce oxidative stress, inflammatory signaling, and metabolic imbalance, all of which may facilitate genomic instability and malignant transformation. However, the molecular mediators linking hyperglycaemia to pancreatic cancer progression remain poorly understood.

microRNAs are key post-transcriptional regulators of gene expression and play an essential role in controlling cellular processes such as proliferation, differentiation, and stress responses. Aberrant microRNA expression has been implicated in pancreatic cancer; nevertheless, the impact of chronic hyperglycaemia on microRNA-mediated regulatory networks in pancreatic cells is still poorly characterized.

This study aimed to evaluate the impact of chronic hyperglycaemia on microRNA expression patterns, cell viability, and DNA damage in human normal pancreatic duct and pancreatic cancer cells.

The study was performed on human pancreatic ductal adenocarcinoma cells (PANC-1 and MIA PaCa-2) and normal pancreatic duct epithelial cells (HPNE). To induce a state of hyperglycemia, D-glucose was added to the standard medium in an amount that increase its final concentration 2-3 fold, and cells were cultured for 28 days. HPNE cells were maintained in a standard medium containing 5.5 mM glucose, defined as normoglycemia (NG), and in 11 and 16.5 mM glucose, which was defined as hyperglycemia (HG). PANC-1 and MiaPaCa2 cells were cultured in a standard medium containing 25 mM glucose (NG), and in 50 and 75 mM glucose (HG).

Cell viability was measured every 7 days using trypan blue staining and Annexin V/PI staining. DNA damage was assessed using the alkaline version of the comet assay. Global microRNA expression profiling was performed using TaqMan™ Array Human MicroRNA A+B Cards v3.0, covering 754 microRNAs. Bioinformatic analyses employing Arena-Idb, MIENTURNET, miRDB, miRNet, miRWalk, TargetScan and Diana Tools were used to predict target genes and enriched biological pathways associated with hyperglycaemia-responsive microRNAs.

MicroRNA profiling revealed extensive alterations in microRNA expression across all cell types, with 87 dysregulated microRNAs identified in MIA PaCa-2 cells, 51 in PANC-1 cells, and 68 in HPNE cells. Bioinformatic analyses of predicted targets of hyperglycaemia-responsive microRNAs demonstrated cell type-specific pathway enrichment. In normal HPNE cells, enriched pathways included cell cycle, p53, PI3K-Akt and MAPK signaling, as well as protein processing in the endoplasmic reticulum and ubiquitin-mediated proteolysis, suggesting potential involvement in genomic instability and early carcinogenic events. In contrast, in PDAC cell lines (PANC-1, MIA PaCa-2) enriched pathways were predominantly related to cancer progression, including AMPK, FoxO, focal adhesion, regulation of the actin cytoskeleton and proteoglycans in cancer, indicating possible links to EMT-associated processes (epithelial-mesenchymal transition), invasive potential and adaptive stress responses. Notably, two microRNAs, were consistently dysregulated in response to hyperglycaemia irrespective of cell type and were predicted to regulate 36 target genes involved in DNA damage-repair, cell cycle control and EMT.

Chronic exposure to hyperglycaemia did not affect the viability of either normal or cancer cells. However, it significantly increased the levels of endogenous and oxidative DNA damage in normal pancreatic duct cells, but not in cancer cells, suggesting potential early genomic destabilization under chronic hyperglycaemia conditions.

Our findings demonstrated that chronic hyperglycaemia markedly alters microRNA expression in pancreatic duct normal and cancer cells. As revealed by *in silico* analysis hyperglycaemia-responsive microRNAs represent epigenetic mediators of key processes in PDAC development and progression, including DNA damage repair, cell cycle control and EMT.

Rubus fruticosus L. leaves – an insight into phytochemistry and biological activity

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Medicinal plants have played a crucial role in healthcare for centuries, and many modern medications are plant-based or derived from phytocompounds. Blackberry (*Rubus fruticosus* L.) leaves, a primary by-product of blackberry harvesting, are underutilized as a medicinal plant material despite their recognized potential in traditional medicine and their inclusion in the Polish Pharmacopoeia [1]. This review summarizes the current knowledge on *R. fruticosus* leaves, focusing on their phytochemistry, confirmed biological activities, therapeutic potential, and the limitations to their wider medicinal use. The work is based on 38 papers from 1980 to 2025, sourced from five major databases.

R. fruticosus L. (Rosaceae), also known as blackberry, is a fruit-bearing shrub of significant agricultural value, cultivated in temperate regions of Europe, Asia, and North America. Traditionally, its leaves are used as astringents and antidiarrheal agents for gastrointestinal diseases, as well as for treating diabetes, upper respiratory tract infections, and skin disorders. Numerous studies have aimed to identify the phytoconstituents of blackberry leaves, validate their traditional uses, and explore novel therapeutic applications. Phytochemical analyses, primarily using high-performance liquid chromatography coupled with mass spectrometry, have revealed that the leaves are rich in polyphenols, including tannins (mainly ellagitannins), flavonoids, and phenolic acids, as well as fatty acids and terpenoids. Biological studies, both in vitro and in vivo (animal models), have highlighted antioxidant, lipid-lowering, antidiabetic, neuroprotective, anti-inflammatory, antimicrobial, and skin-regenerative properties of the leaves.

While much of the research has focused on the antioxidant and antimicrobial activity of *R. fruticosus* leaves, studies have largely been limited to cell-free in vitro models. Only a few in vivo animal studies have supported the antidiabetic, lipid-lowering, and neuroprotective effects of the leaves. Evidence for their use in gastrointestinal disorders, their primary traditional application, is also insufficient. Although the existing data is promising, further in-depth investigations, both in vitro and in vivo in animal models and in human trials, are required to elucidate the molecular mechanisms underlying the activity of blackberry leaves, confirm their pharmacological potential, and assess their clinical efficacy. Comprehensive phytochemical characterization, particularly of tannins, as likely their main active compounds, and the identification of activity markers for quality control are also needed to enhance the role of *R. fruticosus* leaves in modern medicine.

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Impact of gallic acid metabolites on EMT modulation in colorectal cancer

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Background

Colorectal cancer (CRC) is highly lethal, largely due to its strong metastatic potential and its ability to modulate the tumor microenvironment [1]. Furthermore, current treatment methods remain inadequate for advanced CRC, which is unfortunately the stage at which most patients (approximately 62%) are diagnosed [2,3]. Therefore, it is crucial to pursue novel therapeutic strategies. While gallic acid is well-known for its anti-cancer properties, it is its derivatives that primarily circulate in human plasma [4]. Additionally, these compounds have not been investigated in the context of colorectal cancer. Consequently, we examined how nine of phase II gallic acid metabolites affect processes related to epithelial to mesenchymal transition (EMT) across colorectal cancer cell lines characterized by varying levels of invasiveness.

Purpose

The aim of the study was to examine how nine selected metabolites of gallic acid influence the EMT-related processes in colorectal cancer cells, to determine whether their potential effects differ based on the invasiveness of the specific cell line, and to define the underlying mechanism of their action.

Methods

The study was conducted using two colorectal cancer cell lines, LS180 and LoVo. Cell viability was assessed using the SRB assay for four representative compounds, and IC20 values were determined. Subsequently, cells were treated with the selected concentrations and their invasive potential and migration toward type I collagen were evaluated using a Transwell system. In addition, MMP-7 protein levels in conditioned media were measured by ELISA. Cell viability was also assessed in a normal colon epithelial cell line (CCD 841 CoN).

Results

Gallic acid metabolites significantly impaired invasion and migration in LoVo cells, with sulfated and glucuronidated forms showing differential inhibitory potency. These effects coincided with reduced MMP-7 protein levels. In contrast, no statistically significant changes in invasion, migration, or MMP-7 levels were detected in the LS180 cell line. Survival curves obtained for normal colon epithelial cells revealed no detectable effects of the compounds on cell viability.

Conclusion

Gallic acid metabolites exhibit the ability to limit EMT-related processes in invasive colorectal cancer cells. One potential mechanism underlying their activity is the reduction of extracellular matrix degradation. The tested compounds do not exert a significant effect on cell lines with low invasive potential and likewise show no significant effects in normal colon cells.

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Therapeutic potential of targeting free fatty acid receptors in pancreatic disease

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Free fatty acid receptors (FFARs), a distinct subset of G protein-coupled receptors, play a pivotal role in preserving immune and metabolic homeostasis[1]. The four primary subtypes, FFAR1, FFAR2, FFAR3 and FFAR4, are critical to pancreatic physiology, where they modulate insulin secretion, glucose metabolism, and inflammatory responses [2,3]. Consequently, the dysfunction or stimulation of these receptors is linked to the pathogenesis of significant pancreatic disorders, such as acute pancreatitis (AP), pancreatic cancer (PC), and both type 1 (T1D) and type 2 diabetes (T2D) [3,4].

FFAR roles vary by pancreatic pathology. In AP, FFAR1, FFAR3, and FFAR4 exert a protective influence via anti-inflammatory mechanisms [2,5–7]. In PC, their functions are more nuanced: FFAR1 inhibits cell migration, while FFAR3 restricts proliferation [4,8]. Conversely, reduced FFAR2 expression correlates with tumor progression [5]. FFAR4 presents a paradox, simultaneously promoting metastasis and inducing apoptosis [4]. In diabetes, FFAR2 and FFAR4 regulate glucose independently of insulin in T1D, whereas all four subtypes contribute to glycemic regulation in T2D [3,9–11].

While FFARs show clinical promise, their precise mechanisms in pancreatic disease are still being mapped. Current research focuses on signaling pathways, ligand identification, and drug safety. Together, these efforts highlight FFARs as key targets for novel pancreatic therapies.

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Lipid composition changes in perivascular adipose tissue upon high-fat diet induced obesity: The role of stearoyl-Coenzyme A desatuase 1

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Perivascular adipose tissue (PVAT) surrounds blood and lymphatic vessels. Around aorta, PVAT exhibits heterogeneity resulting in diverse, region-dependent phenotypes in the thoracic aorta (TA) or abdominal aorta (AA). As an endocrine organ, PVAT releases bioactive compounds that regulate vessel function and, due to its direct contact with the adventitia, enables bidirectional signaling with the vessel wall. Dysregulation of PVAT-vascular wall cross-talk leads to lipid metabolic disorders i.e. type 2 diabetes, obesity, atherosclerosis [1]. One of the crucial factors involved in lipid metabolism is stearoyl-CoA desaturase 1 (Scd1), an enzyme catalyzing the synthesis of monounsaturated fatty acids from saturated fatty acids in the process of *de novo* lipogenesis.

Despite its prominent role in maintaining vascular homeostasis, certain aspects regarding PVAT physiology remain understudied and new analytical tools are required to evaluate the PVAT lipid profile and to better understand its relationship with vascular function. In this work, we combined Raman spectroscopy with gene expression analysis to characterize the lipid composition and phenotype of TA and AA PVAT upon high-fat diet (HFD) and in an *in vitro* study of PVAT adipocytes.

Based on differences in lipid composition, expressed by spectroscopic marker, lipid unsaturation degree (I_{1660}/I_{1444}) and gene expression profile, we distinguished the phenotypes of TA and AA PVAT. With Raman imaging of PVAT primary adipocytes, we confirmed similar differences in lipid unsaturation on a single-cell level. In addition, we presented that HFD contributes to a decrease in lipid unsaturation by affecting *de novo* lipogenesis. Finally, we investigated the role of Scd1 in PVAT-derived differentiated adipocytes and tested whether its inhibition influences PVAT lipid profile and phenotype alterations.

This work presents spectroscopic and molecular characterization of heterogeneous PVAT, indicating Scd1 as a significant factor of PVAT's lipid composition, which is considered as a promising therapeutic target for the treatment of obesity.

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The effects of poly(ethylene terephthalate) (PET) nanoparticles on functions of the selected components of the human hemostasis system *in vitro*

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According to the most recent report published by Plastics Europe, global plastic production increased from 400.4 million tons in 2020 to 430.9 million tons in 2024. Poly(ethylene terephthalate) (PET) accounts for approximately 6.2% of total production, corresponding to 26.72 million tons. Literature provides information about nanoplastic particles that may pose significant risks to human health. Nanoparticles within the size range of 1-100 nm are able to penetrate organisms through the skin, digestive system and respiratory system. According to recent data, PET particles are among the predominant particles detected in the respiratory tract. Despite the confirmed presence of PET nanoplastics in human tissues and bloodstream, the adverse consequences associated with their accumulation remain insufficiently understood.

The aim of this study was to investigate the effect of poly(ethylene terephthalate) (PET) spheric nanoparticles (of diameters about 130-140 nm) on the hemostatic function of human plasma proteins involved in the coagulation cascade. Human plasma and isolated plasma protein preparations were incubated with PET nanoparticles at concentrations ranging from 0.05 to 50 µg/ml. The effect of PET nanoparticles was assessed by determining the thrombin-induced plasma clotting time, the kinetics of isolated fibrinogen polymerization, plasma clot formation and fibrinolysis (CFF) rate, as well as by assessing enzymatic activities of thrombin (the coagulation factor IIa) and the coagulation factor Xa.

PET nanoparticles slightly decreased the thrombin time (TT) when compared to the control plasma (not treated with the PET nanoparticles). In addition, the PET nanoparticles (at a concentration of 50 µg/ml) reduced the rate of fibrin clot formation by about 55%. Fibrinolysis efficiency increased by 25% at the same concentration. Incubation of thrombin with nanoparticles caused a decrease in the maximal velocity of fibrinogen polymerization by 55% at the highest concentration and by 25% at a concentration of 5 µg/ml. The maximal velocity of fibrinogen polymerization in samples containing fibrinogen treated with the nanoparticles decreased by about 25-50% (at concentrations of 5-50 µg/ml, respectively).

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Cerebellar Transcriptomics in Male Offspring: Impacts of Paternal Age and *In Vitro* Culture

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Advanced paternal age (APA) is associated with decreased semen quality, difficulty achieving pregnancy, and increased health risks for offspring [1]. Assisted reproductive technologies (ART) are increasingly utilized among individuals of advanced paternal age, yet the combined impact of APA and *in vitro* culture (IVC) on neurodevelopmental programming remains poorly understood. This study aimed to evaluate: (1) the transgenerational effect of APA on the cerebellar transcriptome of male offspring in the F1 and F3 generations; (2) the effect of APA on IVC-derived offspring; and (3) the isolated effect of IVC on offspring neurodevelopment.

A transgenerational C57BL/6 mouse model was employed to investigate the effects of paternal age and periconception environment on offspring cerebellar development. For transgenerational studies, adult male offspring (3-5 months) from APA sires (>12 months) and young paternal age (YPA) controls (3 months) were analyzed in the F1 and F3 generations. To assess environmental influences, embryos were conceived and developed under three distinct conditions: (1) natural pregnancy (NP), (2) *In vivo* conception with embryo transfer at the blastocyst stage (ET), and (3) *In vitro* culture for 96 hours followed by embryo transfer (IVC+ET). Cerebellar tissue was dissected from adult male offspring, total RNA extracted, and a panel of 25 neurodevelopmental and epigenetic target genes quantified using the QIAcuity OneStep Advanced EvaGreen RT-dPCR system (Qiagen), with Pgk1 as the validated reference gene. Differential expression was analyzed via volcano plots and Welch's t-tests.

We identified three distinct trajectories determined by the periconception environment. Under natural conception, APA F1 offspring exhibited focal synaptic deficits (Nrxn1↓, Sst↓), resolved by F3—indicating transgenerational compensation in native environments. Under *in vivo* conception with embryo transfer, we observed glio-neuronal network collapse centered on lipid signaling disruption (Fabp5↓, Ptgds↓) with silencing of core connectivity genes (Nrxn1, St8SIA, Ahi1), implicating glial health as a critical ART outcome determinant [2]. Under culture stress (IVC), embryos entered epigenetic/morphogenic redirection: simultaneous upregulation of Hdac3 and Dnmt3a with dysregulated retinoid signaling (Cyp26b1↑, RxrA↓)—conflicting epigenetic programs activated without coordination, representing active developmental redirection at the interface of paternal epigenetics and early embryo stress [3].

This work reveals that the early embryo environment determines both the nature of paternal age-related risk and transgenerational adaptation capacity. The same aged sperm produces different outcomes depending on periconception conditions—from reversible synaptic deficits in nature to irreversible epigenetic changes in culture [4]. For ART Practice: Risk stems from specific procedures; stark differences between ET and IVC+ET highlight the need to optimize culture conditions, particularly components affecting retinoid and lipid signaling [5]. For Clinical Screening: Consistent disruption of lipid and retinoid pathways positions them as promising biomarkers for assessing embryonic health and predicting neurodevelopmental outcomes in ART offspring. For Intervention: Bolstering glial health and reducing pre-implantation stress—through optimized media or reduced culture duration—may mitigate transcriptional dysregulation. Findings suggest paternal age-specific ART optimization is needed to safeguard offspring neurodevelopment.

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Anti-inflammatory potential of isoflavone-rich plant extracts in prostate model *in vitro*

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Chronic inflammation is increasingly recognized as a significant factor in the pathogenesis of prostate cancer and benign prostatic hyperplasia, influencing cell proliferation, tissue remodeling, and local androgen signaling. Interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) are key proinflammatory cytokines regulating the immune response. Interleukin-10 (IL-10) plays a significant anti-inflammatory role, limiting excessive inflammatory responses. Isoflavone compounds, classified as phytoestrogens and found in plants of the Fabaceae family, exhibit biological potential, including immunomodulatory and anti-inflammatory properties. The aim of this study was to evaluate the effect of plant extracts rich in isoflavones on the secretion of IL-6, TNF-α, and IL-10 in human PNT2 prostate epithelial cells stimulated with cytokine mix. Additionally, IL-6/IL-10 and TNF-α/IL-10 ratios were calculated as indicators of the balance between pro- and anti-inflammatory responses.

Analysis of IL-6 levels showed that most of the tested extracts reduced the secretion of this cytokine compared to the inflammatory control. An inhibitory effect was observed at concentrations of 100 µg/mL and 300 µg/mL, with the higher concentration showing a tendency towards stronger inhibition only in two species, indicating a partial dose-response relationship. In the case of TNF-α, a significant reduction in secretion was observed only for selected extracts, with no clear dose-response relationship. At the same time, some of the tested samples demonstrated activity comparable to the short-term effects of dexamethasone. Analysis of the IL-6/IL-10 and TNF-α/IL-10 ratios revealed that selected isoflavone-rich extracts shifted the immune response toward an anti-inflammatory effect, manifested by a reduction in proinflammatory cytokines while maintaining or increasing IL-10 levels. The lowest cytokine ratios suggested particularly favorable modulation of immune balance.

The obtained results indicate that plant extracts high rich in isoflavones revealed promising anti-inflammatory potential in the context of prostate diseases associated with chronic inflammation. However, further mechanistic and in vivo studies are necessary to confirm the observed biological effects.

Molecular Alterations in Urinary Bladder Cancer: Telomere Length and Mitochondrial DNA Copy Number

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Urinary bladder cancer (BC) remains an important clinical problem and is one of the most frequently diagnosed malignancies of the urinary tract [1]. Among the molecular features potentially associated with carcinogenesis, telomere length and mitochondrial DNA (mtDNA) copy number are regarded as particularly relevant [2,3]. Telomeres are repetitive DNA sequences located at chromosome ends that protect genomic stability, and their shortening may reflect genomic instability [2]. Changes in mtDNA copy number may also reflect mitochondrial dysfunction and disturbed cellular homeostasis [3]. Since both parameters have been implicated in carcinogenesis, their evaluation may provide additional insight into BC-related molecular changes.

Tumor tissues, adjacent non-tumor tissues, and blood samples obtained from patients with BC were examined. Telomere length and mtDNA copy number were determined by qPCR method, and the findings were further analyzed in relation to selected clinical and lifestyle-related factors of the examined patients.

The results demonstrated differences between tumor and non-tumor tissue in both telomere length and mtDNA copy number. In general, BC tissues showed a tendency toward reduced telomere length and altered mtDNA copy number compared with the corresponding non-tumor tissues. Variability in these parameters was also observed between patients, suggesting possible associations with individual clinical characteristics and risk factors. Overall, the findings suggest that telomere length and mtDNA copy number may be related to molecular alterations accompanying BC and may support a better understanding of the biological complexity of this disease.

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The effect of nitric oxide synthase on doxorubicin-induced formation of polyploid giant cancer cells in breast cancer cell line

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Doxorubicin remains a cornerstone of breast cancer chemotherapy [1,2]; however, its clinical efficacy is frequently limited by the development of treatment resistance [3,4]. Recent studies have identified nitric oxide synthase (NOS) as a key factor influencing cancer cell responses to chemotherapeutic agents [5,6].

In this study, we investigated the molecular dynamics underlying the formation of polyploid giant cancer cells (PGCCs). The MDA-MB-231 breast cancer cell line was subjected to doxorubicin-induced stress to promote PGCC formation. The presence of the PGCC phenotype was confirmed through morphological assessment using microscopy and quantitative ploidy analysis by flow cytometry. Subsequently, NOS1 gene expression was evaluated at both the transcriptional and translational levels using quantitative real-time PCR (qRT-PCR) and Western blot analysis, respectively.

This study is expected to clarify whether NOS1 expression correlates with the emergence of the PGCC phenotype following doxorubicin treatment. Identifying such an association would provide preliminary insight into the potential involvement of nitric oxide signaling pathways in chemotherapy-induced cellular adaptation.

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Macrophage-related genes *ALK*, *DDR2* and *FLT4* associate with OS and DFS in metastatic colorectal cancer patients: identification of new biomarkers through integrative bioinformatic analysis

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Colorectal cancer (CRC) is one of the most common malignancies worldwide and remains one of the leading causes of cancer-related mortality[1], [2]. Increasing evidence indicates that tumor-associated macrophages (TAMs), plays a crucial role in tumor progression, immune modulation and metastatic progression [3]. Identification of molecular markers associated with macrophage-related signaling pathways may therefore provide new diagnostic and prognostic biomarkers in metastatic CRC.

This study aimed to identify genes associated with macrophage-related biological processes involved in metastatic CRC. Gene lists were obtained from the Metastatic Colorectal Cancer dataset (MSK, Cancer Cell 2018) available in cBioPortal. Gene expression and clinical data were retrieved from the TCGA Firehose Legacy colorectal adenocarcinoma cohort. Expression values as mRNA z-scores were integrated with clinical data to create a dataset including only patients with complete molecular and clinical information. Functional annotation was performed using DAVID and KEGG to identify macrophage-related processes including macrophage activation, macrophage colony-stimulating factor signaling pathway and response to macrophage colony-stimulating factor. Hierarchical clustering and heatmap visualization were conducted using Cluster 3.0 and visualized by Java TreeView. Kaplan-Meier analysis was used to evaluate the prognostic significance of selected genes for overall survival (OS) and disease-free survival (DFS).

In silico analysis identified *ALK*, *DDR2* and *FLT4* as genes associated with macrophage-related signaling pathways in metastatic CRC. High *ALK* expression was significantly associated with shorter OS (65.8 vs. 124.2 months; log-rank p = 0.017). High *DDR2* expression was associated with reduced DFS (log-rank p = 0.0073). The median DFS was 55.12 months in the high-expression group and was not reached in the low-expression group. High *DDR2* expression increased the risk of recurrence (HR = 1.79; 95% CI: 1.17-2.75). High *FLT4* expression was associated with shorter DFS (log-rank p = 0.0114). The median DFS was 59.17 months in the high-expression group and was not reached in the low-expression group, with increased recurrence risk (HR = 1.73; 95% CI: 1.13-2.64).

Elevated expression of macrophage-related genes *ALK*, *DDR2* and *FLT4* was associated with poorer clinical outcomes in CRC. These genes may contribute to tumor progression and represent potential diagnostic and prognostic biomarkers in metastatic CRC.

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miRNA-126 impact Human Induced Pluripotent Stem Cells derived myoblast differentiation

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Skeletal muscle regeneration is a complex biological process regulated by myogenic progenitor cells and post-transcriptional mechanisms of gene expression, including microRNAs (miRNAs). Dysregulation of miRNA activity may impair muscle repair and differentiation, highlighting the need for approaches that enable targeted modulation of myogenic pathways. Human induced pluripotent stem cells (hPSCs) derived myoblasts represent a valuable source myogenic cells with potential applications in regenerative research.

The purpose of this study was to evaluate whether selected miRNAs, i.e. miRNA-126 can enhance human induced pluripotent stem cells (hPSCs) derived myoblasts diffentiation. MiRNA-126 is a key regulator of skeletal muscle homeostasis and regeneration, particularly through its control of angiogenesis and endothelial–muscle cross-talk. It is highly enriched in endothelial cells and modulates signaling pathways that control blood vessel formation, repair, and inflammation.

Human pluripotent stem cells derived myoblasts were cultured under defined conditions and subsequently transfected with miRNA-126.The effects of miRNA-126 overexpression on cell proliferation, apoptosis, differentiation, and the expression of myogenic markers were evaluated using molecular and cellular biology techniques, including qRT-PCR and immunofluorescence staining.

Our findings demonstrate that modified miRNA-126 impact the differentiation potential of human induced pluripotent stem cell–derived myoblasts in vitro. These results highlight the potential of targeted miRNA-based strategies to optimize stem cell–derived myogenic models and support future applications in skeletal muscle regenerative research.

Effect of *Pistacia vera* Leaf Extract on Sensitization of MCF-7 Breast Cancer Cells to Ionizing Radiation

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The increase in life expectancy, progressive industrial development, and growing environmental pollution contribute to the rising incidence of cancer. Epidemiological data indicate that in highly developed countries, higher cancer incidence is observed, which is associated, among others, with improved disease detection and broader access to screening programs. In contrast, in less developed countries, limited access to modern diagnostic methods and effective treatment results in higher cancer-related mortality.

Despite significant advances in oncological therapy, the World Health Organization (WHO) reported that in 2015, cancer accounted for approximately 8.8 million deaths worldwide, and projections indicate that this number may exceed 13 million annually by 2030. These data emphasize the urgent need to seek new and more effective therapeutic strategies [1].

Naturally derived compounds represent a valuable source of potential anticancer agents due to their broad spectrum of biological activity and ability to modulate the effectiveness of standard therapeutic approaches. Their application in radiotherapy to enhance the sensitivity of cancer cells to ionizing radiation could significantly improve the efficacy of radio-oncological treatment [2].

The aim of this study was to evaluate the effect of *Pistacia vera* leaf extract on MCF-7 breast cancer cells exposed to ionizing radiation. The experiments were conducted on cells treated with the extract alone and in combination with ionizing radiation.

Based on the MTT assay, concentrations corresponding to the IC_{50} value were determined. The levels of reactive oxygen species (ROS) and the extent of DNA damage were assessed based on the level of phosphorylated histone H2AX (p-H2AX) and the expression of Rad51 protein, which is involved in DNA repair processes. Additionally, the levels of antioxidant enzymes, including catalase, SOD1, SOD2, GPX4, and the transcription factor NRF2, a marker of oxidative stress response, were determined. Protein expression was analyzed using Western blot.

The obtained results indicate a moderate antioxidant potential of the studied extract, which may be associated with the presence of antioxidant compounds such as tannins and flavonoids. It was observed that treatment with the extract led both to an increase in the number of DNA double-strand breaks and to enhanced DNA repair processes. Moreover, under these conditions, a decrease in the levels of selected antioxidant enzymes was noted.

In conclusion, the results suggest that *Pistacia vera* leaf extract may influence the response of breast cancer cells to ionizing radiation through modulation of oxidative stress and DNA repair mechanisms. However, the observed effects require further detailed studies to clearly define the mechanism of action and potential clinical relevance.

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The effect of genipin on lipid peroxidation in human blood platelets, *in vitro*

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Blue food colorants are widely used to improve the sensory properties of processed foods, and their safety is still being evaluated. Recent reviews indicate a growing interest in natural alternatives, including genipin, a non-glycosidic iridoid obtained mainly from the fruits of *Gardenia jasminoides* and *Genipa americana,* which acts as a precursor of blue pigments and a natural crosslinking agent for food and biomaterial related applications [1,2]. Although genipin-based colorants such as gardenia blue have undergone genotoxicity testing, available toxicological data-especially for blood cells remain limited and sometimes equivocal [3]. In addition, recent *in vitro* studies suggest that genipin can modulate haemostatic properties of blood components without overt cytotoxicity toward human platelets [4].

Blood platelets are central to haemostasis and an important source and target of reactive oxygen species. Oxidative stress and lipid peroxidation products can accumulate in platelets during storage or pathological conditions and are thought to influence platelet activation and interactions with the vascular wall [5]. Lipid peroxidation in biological samples is commonly monitored by the thiobarbituric acid reactive substances (TBARS) assay, which, despite certain analytical limitations, remains a widely used indicator of oxidative damage to membrane lipids [6].

The aim of this *in vitro* study is to evaluate the effect of genipin on lipid peroxidation in human blood platelets. Washed platelets obtained from healthy volunteers will be incubated with increasing concentrations of genipin under basal conditions, in the presence of an oxidant system inducing oxidative stress, and after simulation with thrombin to mimic activated platelets. Lipid peroxidation will be assessed by the TBARS assay.

The planned experiments are expected to provide new information on the interaction between genipin and human blood platelets at the level of oxidative processes. These data may support future safety evaluation of genipin as a potential food colorant and as a component of genipin-crosslinked biomaterials that may come into contact with blood.

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Impact of glucose availability and OGT inhibition on the expression of glycolytic enzymes in HT-29 colorectal cancer cells

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Colorectal cancer is the third most frequently diagnosed malignancy worldwide and the second leading cause of cancer-related mortality. Environmental factors strongly correlated with its increasing incidence include population aging, unhealthy diet, and obesity. One of the key features of colorectal cancer progression is metabolic reprogramming of cancer cells, particularly the enhancement of glycolysis despite the presence of oxygen, known as the Warburg effect. Increased glucose utilization enables cancer cells to rapidly generate energy as well as biosynthetic precursors required for intensive proliferation. Glucose availability within the tumor microenvironment plays an important role in regulating the expression of glycolytic enzymes and metabolic pathway activity. Glucose availability influences the activity of the hexosamine biosynthetic pathway and UDP-GlcNAc levels, which may modulate O-GlcNAcylation catalyzed by OGT. This modification affects the stability, localization, and activity of numerous regulatory proteins, including key glycolytic enzymes such as hexokinase 2 (HK2) and pyruvate kinase M2 (PKM2), which play an important role in the metabolic adaptation of cancer cells. Increasing attention in cancer research is being given to three-dimensional (3D) culture models, which better reflect tumor architecture, oxygen and nutrient gradients, and cell-cell as well as cell-extracellular matrix interactions compared to conventional two-dimensional (2D) cultures.

The aim of this study was to evaluate the effect of glucose availability and OGT inhibition on the protein expression levels of selected glycolytic enzymes (HK2, PKM2) in HT-29 colorectal cancer cells cultured in both 2D and 3D models.

The results indicate that alterations in glucose availability and OGT inhibition differentially modulate the expression levels of the analyzed glycolytic enzymes, in a model-depend manner.

These findings suggest a significant role of the glucose-OGT axis in regulating metabolic reprogramming in HT-29 cells and highlight the importance of 3D culture models in studying mechanisms of metabolic adaptation in cancer.

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