ORAL PRESENTATION ABSTRACTS

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Extraskeletal osteosarcoma description of an unprecedented immunohistochemical profile

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Extraosseous osteosarcomas are a rare malignant mesenchimal tumors arising in non skeletal soft tissues while maintaining the exact same characteristics of bone osteosarcoma and microsocpically is indistinguishable from it producing osteoid, immature bone, and chondroid matrix. Extraskeletal osteosarcomas were described in animals in several soft tissues as breast, bladder, prostate, muscles and in other organs also in association as a progression of preexisting other forms of tumors. Aim of our study is to describe aspects of immunohistochemical reactivity towards selected markers little investigated to date and in comparison with some cases of primary osteosarcomas of the skeletal. our study envisaged a comparative immunohistochemical investigation mainly against collagen 1, collagen 3, RUNX2, Karyopherin alpha 2 and the results will be described in detail and commented in this scientific context.

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Prospects for the use of amaranth phytomass in fodder production

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One of the promising fodder crops is amaranth, which has a unique chemical composition: in terms of absolutely dry weight contains, %: crude protein 15.6-16.75 (in leaves up to 30), fat - 2.4-2.8, fiber - 16.0-21.7, calcium 2.1-2.6, phosphorus 0.2-0.21, carotene 160-200 mg. The inclusion of amaranth in poultry diets has a positive effect on productivity, safety and physiological condition. Vyshtakalyuk A.S., Khirug S.S. found that the use of amaranth phytomass in its pure form causes some deterioration in the condition of birds. This can be caused by excess fiber levels, an excess of certain amino acids in the diet, or the presence of anti-nutritional substances in the plant. However, to date, information on the content of anti-nutritional substances in the green mass of amaranth is contradictory. One of the priority areas in the production of feed additives is the bioconversion of plant materials using enzymatic hydrolysis. We have found that tannins, phytic and oxalic acids, and nitrates accumulate in the phytomass of amaranth variety Giant (harvest of 2022). In the course of the work, a multi-enzyme complex and optimal modes of bioconversion of amaranth green mass were selected, which make it possible to reduce the content of anti-nutritional substances.

The work was carried out within the framework of the Russian Science Foundation grant no. 22-76-00062.

Keywords: amaranth, enzymatic catalysis, anti-nutritional substances.

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Waste valorization to produce bacterial cellulose for agricultural applications

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Mulching is a common agricultural practice that protects plants against biotic and abiotic stresses by controlling the microenvironment around them. Polyethylene mulches are mostly used but plastic pollution has made it imperative to look for alternative materials. Biodegradable polymers are an environment-friendly option. Bacterial cellulose (BC) produced by *Gluconacetobacter xylinus*, is a promising material because of its high mechanical strength and biodegradability. However, the cost of commercial production of bacterial cellulose is high. This study aimed to optimize production of bacterial cellulose using waste as substrate. Kitchen and brewery waste were tested for BC synthesis. The medium composition, pH, inoculum size and time of incubation were optimized for BC production. Brewery yeast and tea waste were found to be a good nitrogen source. Produced BC was characterized using FT-IR, SEM and TGA, and compared to BC produced using standard HS medium. The best results were observed using medium containing potato peel hydrolysate, which gave a yield of 63.3 ± 1.2 g/L compared to HS medium that produced 93.4 ± 1.5 g/L.

The utilization of wastes from household and industry for BC production can help in waste valorization and reduce production costs, increasing the use of BC in agricultural applications to replace polyethylene plastic.

Keywords: Mulch films, plastic pollution, biodegradability, biopolymer, cellulose.

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Microbial polymer of poly- γ-glutamic acid (γ-PGA) – past, present, and future

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Natural polymers are produced by a range of prokaryotic and eukaryotic organisms. Microbial polymers have an enormous potential, as they can be produced from renewable resources under controlled conditions. Poly- γ-glutamic acid (γ-PGA) is a bacterial biodegradable and water-soluble polymer which is attracting interest for range of applications. γ-PGA is an extracellular anionic homopolyamide produced by bacteria, especially *Bacillus* species, including generally recognized as safe (GRAS) - *Bacillus subtilis*. γ-PGA is different from other proteins because glutamate is polymerised through the γ-amide linkages via intermembrane enzymatic complex, and thus is synthesized in a ribosome independent manner. γ-PGA can either be composed of only L-glutamic acid residues (γ-L-PGA), only D-glutamic acid residues (γ-D-PGA) or both L and D residues (γ-L/D-PGA). This polymer is non-toxic, non-immunogenic and biocompatible therefore it can be used in a variety of applications.

This talk will give a detailed insights into microbial synthesis of γ-PGA and its physicochemical properties. Additionally, it will provide an updated overview relevant to sustainable and cost-effective production of this biopolymer, with examples of applications in medicine, pharmacy, cosmetics, food as well as in agriculture.

Keywords: γ-PGA, waste valorisation, sustainability, biomaterials, *Bacillus*

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Evaluation of pigment content of Parachlorella kessleri under mixotrophic conditions

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Studies in recent years related to increasing microalgal biomass production have been conducted to obtain value added products like chloropyhlls and carotenoids. Chlorophylls and carotenoids from microalgal biomass have gain attention due to their applications in pharmaceutical, food and biotechnological industries. While obtaining such products from microalgal biomass, cost of organic carbon source is one of the main challenge. The aim of this study was to investigate the effect of cost effective agro-industrial residue 'vinasse' on biomass productivity, chlorophlyll and carotenoid content of *Parachlorella kessleri*. Preliminary studies were conducted under different growth conditions (autotrophic, heterotrophic and mixotrophic). Cell growth that was measured with optical density demonstrated usage of vinasse in growth medium improved growth of *P. kessleri* and cells demonstrated higher optical density values under mixotrophic conditions (OD_{680} 2.9) than autotrophic (OD_{680} 1.2) and heterotrophic conditions (OD_{680} 1.8). Following selection of mixotrophic growth conditions, a Box-Behnken Design was created with the parameters light intensity (3800-5000-7300 lux), light:dark cycle (16h light:8h dark, 12h light:12h dark, 8h light: 16h dark) and vinasse concentrations (5% -8.5%-12 % (v/v)) to investigate the effect of light, light intensity and vinasse concentration on biomass productivity, chlorophyll content and carotenoid content of *P. kessleri*.

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Removal of cytostatics: bleomycin and vincristine by white-rot fungi - the impact of aeration methods on the drugs' elimination efficiency

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Anticancer drugs which are resistant to wastewater treatment pose an environmental hazard. Therefore, to eliminate cytostatics from waters, fungi-based technologies are worth particular attention.

The aim of the study was to determine the influence of different types of aeration: continuous, periodic, and agitation-induced, on the ability of white-rot fungi to remove cytostatic drugs. The selected organisms were: *Trametes versicolor* (strain CB8) and *Hypholoma fasciculare* (strain CB15). Biodegradation tests of bleomycin and vincristine at an initial concentration of 10 mg/L were conducted for 3 days. The degree of drugs removal was assessed using Ultra-Performance Liquid Chromatography combined with Mass Spectrometry, preceded by Solid Phase Extraction.

The results showed that in the fungi-mediated biodegradation process it was possible to remove up to 96% of vincristine within just 3 days. When it comes to bleomycin, especially agitation-induced aeration resulted in its good removal efficiency at the level of 71% (after 3 days only), presumably due to shaking enhancing both air supply and mycelium-xenobiotic contact.

Such promising results inspire further research into the application of fungi in the elimination of pharmaceuticals.
The study was financed by the National Science Centre, Poland: UMO-2020/37/N/ST8/01077.

Keywords: basidiomycetes; biodegradation; pharmaceuticals

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Multidisciplinary Approach in Medicinal Biotechnology and its Application in the Research of Anti-Covid Drugs, Biosensors, and Scaffolds for Regenerative Medicine

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The development of medicinal biotechnology is increasingly influenced by interaction with other fields ranging from computer modeling, through chemistry, to molecular and structural biology, bioengineering, and molecular medicine. Such multidisciplinary approaches are integrated into the common strategy of biotech research.
The lecture presents selected examples of integrated approaches including drug development of potential drugs against SARS CoV-2, biosensor development, and development of materials for regenerative medicine.
Firstly, various targets of the SARS-CoV-2 virus are briefly reviewed. The latest results of the integrated approach consisting of computer-assisted drug design, bioavailability studies, synthesis, enzymatic and *in vitro* tests of bio-activity of new substances that inhibit coronavirus enzymes M^{pro} and PL^{pro}, as well as SARS-CoV-2 virus *in vitro* in infected human cell lines are presented.
The examples of biosensor development are focused on electrochemical biosensors ranging from the screen-printing technology to nano-biosensors applicable in biomedicine and biotechnology.
Polymeric scaffolds for artificial human urethra were studied using molecular modeling of interactions of human integrin domain with polymeric model surfaces combined with experimental evaluation of adhesion and proliferation of epithelial cells on polymer films.

The support by grants APVV-18-0420, APVV-PP-COVID-20-0010, and APVV-21-0108 is acknowledged.

Keywords: biosensors, drug design, M^{pro}/PL^{pro} SARS-CoV-2 inhibitors, polymeric scaffolds

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Omics Approaches to Study Anorexia Nervosa

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Anorexia nervosa is a multifactorial disorder characterized by severe weight loss and distorted body image. Despite extensive research, the biological underpinnings of anorexia nervosa remain poorly understood. This study highlights the significance of employing omics approaches, such as genomics and metabolomics, to unravel the molecular mechanisms involved in this complex disorder and identify potential therapeutic strategies.
Genomic analyses were conducted using a panel of 163 genes and 730 single nucleotide polymorphisms (SNPs) for DNA sequencing. Additionally, a panel of 52 molecules including amino acids, fatty acid amides, lipids, toxins, endocannabinoids, and inflammation markers was analyzed using metabolomic approaches.
Several interesting genetic variants and polymorphisms associated with anorexia nervosa were identified using the polygenic risk score for susceptibility analysis. Furthermore, significant metabolites were discovered using algorithms for biomarker discovery, shedding light on the metabolic perturbations associated with the disorder.
Through our findings, we are developing and validating genomic and metabolomic panels that contribute to a better understanding of anorexia nervosa's molecular mechanisms. Moreover, these results provide insights into the potential use of natural molecules for the development of innovative formulations that may regulate appetite and hold promise for novel therapeutic interventions.

Keywords: Anorexia Nervosa, Omics Approaches, Genomics, Metabolomics, Molecular Mechanisms

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Long Reads Sequencing for the Diagnosis of Eye Diseases: The Experience of FLG and RPGR Genes

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Accurate diagnosis of eye diseases is challenging due to low-complexity and highly homologous regions in genes such as FLG and RPGR. Traditional sequencing methods often fail to provide comprehensive coverage and accurate variant annotation. The study of many genes presents low-complexity regions, and of specific chromosomal regions, in which massive arrays of tandem repeats predominate. These limitations called for the development of new sequencing techniques: the Third-generation sequencing (TGS) wich comprises long-reads sequencing approaches. In this study, we employed PacBio sequencing to obtain RPGR ORF15 exon and we developed a method for the sequencing of FLG for next sequencing. PacBio successfully captured challenging regions, improved coverage, and accurately detected pathogenic variants. Structural studies aided in variant interpretation. PacBio sequencing has potential as a standard procedure for sequencing genes with low-complexity regions, ensuring accurate variant annotation. Advanced sequencing technologies enhance genetic diagnosis in eye diseases. precise genetic variants identification will permit the implementation of personalized medicine strategies, like gene-therapy, thus providing a new treatment option to patients.

Keywords: Eyediseases,Third-generationsequencing, Low-complexityregions, RPGR, FLG

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G-quadruplex forming sequences modulate basal transcription of a yeast reporter and impact the transactivation by P53 family proteins

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Local DNA structures play an important role in various biological processes. A very thermodynamically stable four-way structure formed in G-rich sequences G-quadruples (G4) is increasingly being revealed as critical players in DNA metabolism, including the accessibility and activity of promoters. Exploiting a well-defined transcriptional reporter system in yeast, we systematically investigated the impact of various G4 prone sequences on basal and induced transcription. A 22-mer G4-prone sequence (derived from the KSHV virus) and five derivatives that progressively mutate the characteristic guanine stretches were placed upstream of a minimal promoter and adjacent to a P53 response element (RE) in otherwise isogenic strains of luciferase reporter. The group of G4 sequences was analyzed by the thioflavin T binding test and circular dichroism in vitro and then we analyzed the impact of G4 sequences on the yeast reporter system. We have measured the basal activity of the reporter as well as the expression induced by transactivation of the p53 family protein. Wild-type P53, P73 and wild-type P63 isoforms, nine cancer-associated P53 missense mutants and three ectodermal dysplasia P63 alleles were tested. The results show that G4-prone sequences increase basal transcriptional activity proportional to their relative propensity to adopt a G4 structure. Furthermore, G4-prone sequences can cooperate with weaker wild-type P63 and P73 proteins but not with P53. Finally, G4-prone sequences increase the ability to transactivate mutant alleles from the partial function P53 family, indicating the importance of including an evaluation of local DNA structural motifs within promoters to predict the functional impact of disease-associated *TP53* and *TP63* mutations. These results point to the possibility of transcriptional regulation by G4-associated transcription factors and by induction of G4 structure formation within yeast promoters.

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By-products of vitivinicultural origin: reutilization within the era of circular economy

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Grapes are cultivated globally in an area reaching 7.3 million hectares in 2022, while the global wine market is expected to increase to USD 583.77 billion by 2032. However, winemaking produces large quantities of by-products/wastes like grape pomace, wine lees, and vine shoots. Given that the majority of them are considered soil pollutants, their disposal represents a cost for wineries. Furthermore, their use as an energy source is not proposed following the Waste Framework Directive (Directive 2008/98/EC) of the EU, that "waste prevention should be the first priority of waste management and re-use and material recycling should be preferred to energy recovery from waste". In this study, the sustainable exploitation of such by-products for application in winemaking is presented. Some examples are (i) the recovery of several ingredients like ethanol, tartaric acid, phenolic compounds, yeast's cell wall polysaccharides (β-glucans and mannoproteins), etc., and (ii) the production of composites for eliminating wine unwanted compounds (like ochratoxin A and pesticides) and as a fining agent of wines. Finally, the recovery of cellulose fibers may lead to nanocomposite film production. It is for the vitivinicultural sector to switch from the already established linear model to the circular economy model.

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Detection of Novel Adipokines in Bovine Ovaries during Different Reproductive Stages

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This study aimed to examine the gene expression profiles of novel adipokines, namely vaspin, adiponectin, visfatin, and resistin, as well as their corresponding receptors (heat shock 70 protein 5, adiponectin receptor 1, and adiponectin receptor 2), within the bovine corpus luteum (CL) across various stages of the estrous cycle (days 1–2, 3–4, 5–7, 8–12, 13–16, and >18) and pregnancy (months 1–2, 3–4, 5–7, and >7). Reverse transcription polymerase chain reaction (RT-qPCR) was employed to measure mRNA expression levels. These were then normalized using the geometric mean of three reference genes (cyclophilin A, ubiquitin, and ubiquitin C) with consistent expression. Our findings demonstrate the presence and distinct regulation of adipokines in all investigated groups. Notably, vaspin and adiponectin exhibited upregulation during the middle and late phases of the estrous cycle. Conversely, resistin displayed higher abundance during CL regression and the early months of pregnancy. The specific expression patterns of adipokine receptors suggest their involvement in the local mechanisms governing CL function. Further investigation is necessary to elucidate the regulatory mechanisms underlying the diverse local effects of adipokines on the ovarian physiology of cows.

Keywords: ovary function, corpus luteum, gene expression, bovine reproduction

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Proteomic Approaches to Study Chronic Fatigue Syndrome and Post-Covid Syndrome

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COVID-19 is a disease caused by the SARS-CoV-2 betacoronavirus. COVID-19 usually presents with flu-like symptoms, and in severe cases with interstitial pneumonia and respiratory failure. In some patients debilitating symptoms persist for weeks or even months. These persistent symptoms are superimposed on those associated with chronic fatigue syndrome/myalgic encephalomyelitis, which is a serious, long-term illness that affects many body systems. To comprehend the differences between healthy controls and Post-Covid Syndrome patients, a proteomic profiling of the serum proteome was performed. 80 patients and 50 healthy controls were analyzed with a panel of 65 proteins. After the analysis, the data obtained was elaborated with PLS-DA analysis and 33 proteins were identified, which were subjected to a ROC analysis. Five proteins, mostly associated to inflammatory processes, were identified as potential standalone biomarkers: Antithrombin, Sirtuin 1, Natriuretic Peptide B, Hemopexin and Arachidonate 5-Lipoxygenase. These preliminary results could be useful for the screening of Post-Covid Syndrome patients.

Keywords: Post-Covid Syndrome, Chronic Fatigue Syndrome, Proteomics, Biostatistics Analysis

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Biostatistics Approaches to Polygenic Risk Score in Physiological Conditions

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Genome-wide association studies (GWAS) have shown that complex traits are associated with many genetic variants, particularly single nucleotide polymorphisms (SNPs). The Polygenic Risk Score (PRS), in a genetic context, is a complex statistical method based on the multifactorial nature and multigenic properties of traits. This method involves the weighted sum of the number of risk alleles at a series of loci, with weights generally represented by the logarithms of the odds ratios or beta coefficients derived from large-scale, information-rich GWAS. The PRS is an aggregate measure that reflects the cumulative influence of multiple genetic variants on a given trait or condition, highlighting the multifactorial nature of the genetics of such traits.

In this work, we are exploring the use of PRS in physiological conditions, aiming to establish its utility in predicting non-clinical traits, potentially accounting for gene-environment correlations. In this context, PRS has enormous potential, allowing for the adaptation of an individual's lifestyle (diet, physical activity, food supplementation) to reduce or prevent the possible negative impact of observed genetic variants.

Keywords: Polygenic Risk Score, Genetic Variants, Biostatistics

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Biostatistics Approaches to Polygenic Risk Score in Multifactorial Conditions

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tes of America

Genome-wide association studies (GWAS) have shown that complex traits are associated with many genetic variants, particularly single nucleotide polymorphisms (SNPs). The Polygenic Risk Score (PRS), in a genetic context, is a complex statistical method based on the multifactorial nature and multigenic properties of traits. This method involves the weighted sum of the number of risk alleles at a series of loci, with weights generally represented by the logarithms of the odds ratios or beta coefficients derived from large-scale, information-rich GWAS. The PRS is an aggregate measure that reflects the cumulative influence of multiple genetic variants on a given trait or condition, highlighting the multifactorial nature of the genetics of such traits.

In this work, we are using PRS to estimate the genetic propensity towards pathological conditions for which a set of genetic variants associated is known. In particular, we focalized on anorexia and obesity. In our tests, PRS showed promising results, confirming its usefulness in predicting an individual's genetic risk for diseases.

Keywords: Polygenic Risk Score, Genetic Variants, Biostatistics, Anorexia, Obesity

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Autoantibodies in Patients with Autoimmune Retinopathy

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Autoimmune retinopathy (AIR) is a rare blinding disease characterized by the presence of auto-antibodies against retinal proteins. Although the first case report is from 1976, the prevalence of AIR is currently unknown. Moreover, diagnosis of AIR can be difficult due to the overlap of symptoms with hereditary retinal degeneration, and to the lack of standard diagnostic consensus and standard diagnostic tests for the detection of circulating autoantibody against specific retinal antigens. Nevertheless, diagnosis of AIR is fundamental to select patients for the correct therapeutic approach and for available clinical trials. With the aim to develop a new screening test for AIR, we used Western Blotting assay and Dot Blot technique to analyse the serum of 20 patients and 10 controls. Results indicated that Recoverin, Rodopsin, and G protein subunit alpha transducin 2 are good candidates for the screening of AIR.

Keywords: Autoantibodies, Autoimmune Retinopathy, Screening test

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Omics Approaches in Lymphedema

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Lymphedema is a chronic inflammatory disorder characterized by fluid accumulation in the lower, upper, and genital limbs, resulting in edema and fibrosis. It can be primary, caused by genetic mutations, or secondary, resulting from infection, surgery, or trauma. Current genetic tests for primary lymphedema yields negative results in nearly 50% of cases, making further research necessary to unveil additional genes implicated in this condition. In addition, some genes have been found to predispose individuals to secondary lymphedema. In this study, we analyzed 343 patients with lymphedema with an NGS panel comprising 95 genes and 64 SNPs to improve our understanding of the mechanisms underlying lymphedema. We then employed in silico methods to assess the pathogenicity of the identified genetic variants and to investigate natural molecules with the potential to treat or prevent the symptoms of lymphedema. Omics sciences such as metabolomics and proteomics could be other promising approaches to identify new therapeutic or diagnostic targets and support the evidence obtained by genomics.

Keywords: lymphedema, NGS, SNPs, omics sciences

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Bioinformatic Analysis to Study the Relationship between AKR1C Genes and Lipedema

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Lipedema is an autosomal dominant genetic disease that ma-

inly affects women. It is characterized by excess deposition of subcutaneous adipose tissue, pain and anxiety. The genetic and environmental etiology of lipedema is still largely unknown. Although considered a rare disease, this pathology has been suggested to be underdiagnosed or misdiagnosed as obesity or lymphedema. Steroid hormones seem to be involved in the pathogenesis of lipedema. Indeed, AKR1C1, a gene coding for a protein involved in steroid hormones metabolism, was the first gene to be correlated with lipedema. In this study, we employed a molecular dynamics approach to assess the pathogenicity of AKR1C1 genetic variants found in patients with lipedema. Three genetic variants in AKR1C1 were disruptive to the protein's function. The results of this study provide supporting evidence that AKR1C1 may be a key gene in lipedema pathogenesis.

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Transdermal diffusion of enzymatically synthesized oil-derived phloridzin esters from oil in water emulsion

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Phloridzin is flavonoid known for its antioxidant and UV-protective effects on the skin. However, it has limited solubility in both hydrophilic and lipophilic media, therefore it should be derivatized prior to incorporation in final products. Hereby, we applied lipase-catalyzed acylation to produce different phloridzin ester mixtures using coconut and linseed oil as acyl donors and examined their transdermal delivery. The new derivatives were incorporated in oil in water emulsion to examine their diffusion through artificial skin-mimicking Strat-M® membrane using Franz diffusion cell. The transport of esters was successfully modelled by modified Kosrmeyer-Pepas equation. Lag phase, lasting 90-170 min, was detected for all esters. For coconut oil-derived esters process was entirely controlled by diffusion, while for linseed oil-based esters polymer relaxation was observed, as well. Effective diffusion coefficients of medium-chain esters obtained using coconut oil were one order of magnitude higher compared to long-chain ones. Zeta potential of both formulations was constant during six month storage period, indicating their high stability. Obtained results underscore suitability of natural triglycerides application in the production of phloridzin esters for topical products.

Keywords: phloridzin ester, oil, transdermal diffusion

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Osmolytes-based Deep Eutectic Solvents: Innovative Systems for Stabilization of Biomolecules

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Studying nature has proven to be an effective method for deriving inspiration to solve complex problems in fields such as engineering, chemistry, and biotechnology. The process of designing solutions based on nature's principles involves multiple disciplines, and the main challenge lies in identifying the natural functions and applying them to specific engineering contexts. Deep Eutectic Solvents (DESs), neoteric solvents created from naturally occurring compounds, have received significant attention as innovative systems that can mimic the natural environment of biomolecules.

We have recently explored the pool of naturally occurring osmolytes, small molecules produced in cells as a response to external stimuli, and the patterns of their natural distribution to form novel osmolyte-based DES. The prepared DES were further tested for their potential to stabilize a template protein (lysozyme). The results suggest that osmolyte-based DES in all their variability have a potential to stabilize lysozyme stored at various temperatures, in a significantly better and longer-lasting manner than the currently known DES and the standard buffer for lysozyme storage. Based on the results, these new bioorelevant DESs may open new opportunities for action in diagnosis, monitoring, treatment and manufacture across several research fields and market sectors.

Keywords: Deep eutectic solvents, osmolytes, biomolecules, bioinspired solvents

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Genomics Analysis of Chronic Fatigue Syndrome and Post-Covid Syndrome

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Chronic fatigue syndrome (CFS) and are two medical conditions that have attracted increasing attention in recent years. Post-Covid Syndrome, known also as Long Covid, is defined by WHO as the set of long-term health consequences caused by Coronavirus infection. The study of the genetic variants associated with these conditions was conducted by enrolling about 101 patients, ranging in age from 18 to 80 years. After a study of the scientific literature and consultation of

dedicated databases, 494 total unique genes and 232 SNPs known to be associated with Post-Covid and CFS were tested with NGS analysis. These findings could led the way for new strategies for diagnosis and treatment. However, further research is needed to fully understand the impact of genetic factors in the two syndromes and to identify potential therapeutic targets. Thus, genomics represents a promising area of study to improve the management and well-being of CFS and Post-Covid patients.

Keywords: Post-Covid Syndrome, Chronic fatigue syndrome, NGS analysis, Genomics.

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Diagnostic potential of extracellular vesicles in prostate cancer

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Prostate Cancer is the second most common type of cancer among men nowadays. One of the biggest challenges is proper diagnosis and stratification for Active Surveillance (AS) approach. Extracellular Vesicles are membranous nano-sized vesicles released by cells that are potentially interesting material for diagnostical procedures of Prostate Cancer.

The aim of this study was an analysis of the surface markers and miRNA profile of the medium-sized plasma EVs (mEVs) for the potential of distinction between 24 Prostate Cancer patients classified for AS and not classified for AS, based on the histopathological Gleason Score results.

For the diagnostic potential analysis of mEVs for AS stratification, nanoFlow Cytometry analysis of surface markers: CD9, CD81, PSMA and EpCam, and the miRNA profile of mEVs were checked.

The analysis of plasma mEVs revealed significant differences in PSMA+ to PSMA+CD9+ EVs ratio between AS and non-AS patients. MiRNA profiling showed higher miR-99a-5p, miR-125b-5p, miR-145-5p and miR-365a-3p levels in non-AS group.

NanoFlow Cytometry analysis and miRNA profiling of plasma mEVs provides a new alternative to stratify the risk of prostate cancer progression during AS, that need to be confirmed with a bigger group of patients.

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Exploitation of autochthonous L. casei group strains as secondary cultures to control blowing defects in PDO cheeses

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Annually high amounts of cheese are wasted due to the development of late-blowing defects (LBD) in the product, impairing the economic and sustainable performance of dairy industries. Among solutions to reduce LBD in PDO cheeses, a promising alternative is represented by the use of autochthonous secondary bio-protective cultures. In this study, 34 *L. paracasei* and 2 *L. rhamnosus* strains were isolated from a PDO Italian semi-hard cheese, and characterized for their anti-clostridial activity, proteolytic and lipolytic activities and ability to produce volatiles in a model system. Results showed that all strains inhibited *in vitro* the growth of *Cl. sporogenes*, *Cl. beijerinckii*, and *Cl. butyricum*, and 18 of them inhibited at least one *Cl. tyrobutyricum* strain. In addition, some strains exhibited proteolytic activity and developed volatile profiles in the headspace similar to commercial control strains. Thus, to test the efficiency of isolated strains in real conditions, those with medium-strong anti-clostridial activity were selected and directly exploited as adjunct starter cultures for cheesemaking. Different bio-protective microbial pools were tested with the aim to identify the most suitable candidates to control microorganisms responsible for blowing defects in semi-hard cheese without affecting product's typical features.

Keywords: autochthonous cultures; blowing defects; anticlostridial activity; non-starter LAB

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Effect of six fluoroquinolones on the viability of bladder cancer cells in 2D and 3D cultures

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The anticancer properties of fluoroquinolones and their high concentrations in urine may help in bladder cancer therapy. This study aimed to analyze the properties of 6 fluoroquinolones as potential candidates for supportive treatment of bladder cancer. Ciprofloxacin, levofloxacin, norfloxacin, enrofloxacin, moxifloxacin, and ofloxacin were tested on one normal (SV-HUC-1) and two cancer (T24 and HTB-9) urothelial cell lines. In 2D culture, the MTT assay, real-time cell growth analysis, fluorescence and light microscopy, flow cytometry, and molecular analysis were used. In 3D culture, luminescence assays and confocal microscopy were performed. In 2D culture, decreased cell viability, destruction of the actin cytoskeleton, shrinkage of the nucleus, inhibition in the G2/M phase, and increases in the number of late apoptotic cells were observed. These effects were more pronounced in the case of cancer cells. Molecular analysis showed variable expression of studied genes depending on the drug and concentration.

In 3D culture, tested drugs were effective only in the highest tested concentrations, which were accompanied by caspase 3/7 activation and cytoskeleton degradation. This effect was hardly visible in non-cancer cell lines. According to the data, ciprofloxacin has the most promising properties and can potentially support chemotherapy in bladder cancer patients.

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Protein hydrolysate of Tenebrio molitor adults obtained during enzymatic hydrolysis

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Insects are eco-saving protein sources but the high chitin content of adults hinders their utilization, being currently disposed of. Enzymatic hydrolysis (EH) was carried out to extract *Tenebrio molitor* adults' protein testing different commercial enzymes, which the protease from *Aspergillus oryzae* was the most efficient. Optimization of EH determined that an enzyme load of 3195 U g-1 and a liquid:solid ratio of 15 achieved an optimum hydrolysis degree (HD) of 72.2%. In optimum conditions, HD of defatted insect meal was lower (60%) than when raw insect meal (78%) was hydrolyzed, evidencing that the defatting process is not required in *T. molitor* adults' valorization. Hydrolyzed insect meal had lower protein (32%) and higher chitin (47%) contents than the unhydrolyzed biomass (50% and 23%, respectively), demonstrating the effectiveness of EH in protein separation from chitin. It was observed that the protein hydrolysate from *T. molitor* adults had higher digestibility (77.3%) than the larvae (66.2%), pupae (67.4%) and adults (17.8%). This work shows the high efficiency of fungal proteases in separating protein from chitin and obtaining a highly digestible protein-rich product, establishing a novel utilization for *T. molitor* adults and promoting circularity in insects farming.

Keywords: *Tenebrio molitor*, enzymatic hydrolysis, protein hydrolysate

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Specific DNA probes and aptamers for microbial detection using Electrochemical Biosensors

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Aptamers and DNA probes can specifically detect whole cells and biomarkers in food, water, and body fluids. *In silico* design of aptamers using APTERION (ArtaPeptidion) an integrated technological platform, which allow to reduce time and costs, comparing to the classical systematic evolution of ligands by exponential enrichment (SELEX) method, make them useful for the development of label-free electrochemical biosensors. DNA probes have also been successfully used for specific detection of pathogens in food. Both gold and carbon screen printed electrodes were utilized for the analyses with differential pulse voltammetry (DPV). Gold electrodes were functionalized with probes or aptamers modified at 5'end with a thiol group to allow the gold-DNA probe/aptamer binding. Aptamers were designed for the detection of *Escherichia coli* in water samples and tested for sensitivity and specificity using DPV, reaching the sensitivity of 10 cells/mL. A DNA probe specifically designed for the detection of *Listeria monocytogenes* was tested with cyclic voltammetry (CV) and DPV and could detect the DNA of the pathogen at 1 pg/µL. Carbon screen-printed were used for the detection of the spike protein and were able to detect the protein at 0.01 µM in the presence of competing proteins using a specific aptamer.

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A study to evaluate the photodynamic inactivation of *Escherichia coli, Listeria monocytogenes* and *Salmonella enterica*

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Photodynamic Inactivation (PDI) is studied as innovative non-thermal approach to reduce microbial contamination in various fields, including the food sector. PDI operates by utilizing blue light (400-480 nm), oxygen, and either an endogenous or exogenous photosensitizer.

To investigate the effectiveness of blue light generated by LED in the range of 400-460 nm against *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*, an assessment was conducted. Qualitative evaluation aimed to determine the optimal blue light conditions for microbial reduction. Subsequently, a quantitative assessment was performed to estimate the log reduction. Microorganisms were cultivated on agar and liquid media, and they were subjected to different conditions of blue light, as wavelengths, doses, and treatment times. Qualitative findings revealed that the effectiveness of inactivation varied based on the wavelength, light dose, and microbial species when the blue light doses were below 700 J/cm^2. On agar and liquid substrates, the quantitative data demonstrated that a blue light dose exceeding 400 J/cm^2 eliminated the bacteria. Moreover, lower wavelengths of blue light exhibited greater efficacy in reducing microbial populations compared to higher wavelengths. Log reductions ranged from 1 to 7 logs for bacteria grown in agar media and from 2 to 4 logs in liquid media.

It was noted that the efficacy of blue light treatment was dependended on specific microbial species under investigation.

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Immobilization of Feruloyl Esterase from Geobacillus thermoglucosidasius DSM 2542T by CLEA(Cross linking enzyme aggregates) Method

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Feruloyl esterases (FAEs), (EC 3.1.1.73) are a subclass of carboxylic acid esterases, a class of hydrolases that catalyze the formation and breakdown of the ester bond between the ferulate ester groups required for crosslinking between hemicelluloses and hemicellulose-lignin. FAEs have gained great importance in the textile, agriculture, food and pharmaceutical industries, paper and cosmetics industries, and in many industries and medical fields such as obtaining ferulic acid (FA) from industrial wastes.

In this study, FAE of a thermophilic bacterium, *Geobacillus thermoglucosidasius* DSM 2542T, expressed in *Escherichia coli* (*E.coli*) by cloning into pET28a(+) expression vector before, was used (GthFAE) for CLEA immobilization studies. The effects of precipitating agents, crosslinking agent concentration, cross linking time, effect of enzyme concentration, temperature and precipitation time were observed for croslinking. After crosslinking, the stability of immobilized enzyme in organic solvents were observed. The structure of the immobilized enzyme was screened with SEM.

As a result of all optimization studies, it was determined that the enzyme was precipitated in acetonitrile. In the presence of glutaraldehyde, the activity increased as the cross-linking time and the enzyme concentration increased. GthFAE was recovered aproximetaly 100% at the end of the immobilization.

Keywords: CLEA, feruloyl esterase, Geobacillus, immobilization

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Screening Hydrolytic Enzyme Production of Bacterial Strains Isolated from Waste Dumps, Turkey

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Depolymerization of synthetic polyesters via microorganisms has become a focus of research in recent years. Moreover, microbial hydrolytic enzymes such as lipases, amylases, proteases, cellulases, cutinase etc. can be pressed into service for the management of waste produced during industrial applications. In this work we aimed to determine the hydrolytic enzyme production capabilities of some plastic degrading bacteria.

Plastic degrading bacterial strains were isolated in a previous work in our laboratory. After 16S rRNA sequence identification, enzyme production capabilities of the isolates were screened. Amylase, protease, cellulase, lipase, and xylanase were the subject of this study. Plate assays were performed for each enzyme with 0,5-1 % of suitable substrates. Inoculated plates were incubated at 37 °C for 2 days. After incubation, positive strains were determined based on clear zones formed around colonies. Screening results were revealed that 5 of the strains (KA4, TM1, KTM1, petDEG1B, and BKR1) can produce all of the enzymes searched. 13 of the strains can produce at least one of the enzymes screened. Based on these results it can be said that 13 of the strains are useful for waste management. Further analyses both plastic degradation properties and other industrial enzymes will be carried out.

Keywords: Bacteria, hydrolytic enzymes, plastic degradation

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Biopolymeric bacterial cellulose hydrogels for wound management applications

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Acute wounds may turn into chronic type when failing to appoint the correct management intervention. The basic tenets of wound management involve creating optimum condition to facilitate wound healing. Attributing to their distinct properties, hydrogels have attracted vast research interest as advanced wound dressings. Bacterial cellulose (BC) is a biosynthetic hydrogel material that is produced by several bacterial genera including a Gram negative, rod shaped, *Gluconoacetobacter xylinus*. BC can be produced under agitated, stirred, and static fermentation conditions. The properties of BC vary to a great extent with the production technique. In the current study, for biomedical application as wound dressings, BC pellicles were produced under static conditions.

This talk will give an insight into microbial synthesis of BC and its physicochemical properties as a wound dressing matrix. Additionally, it will provide a detailed overview of the production and characterisation of BC-loaded with different healing agents. All the hydrogels were characterized based around their potential wound dressing applications. The antimicrobial, haemocompatibility and cytocompatibility characteristics of these hydrogels were tested *in vitro*. Furthermore, a range of physicochemical characterisation studies were undertaken to evaluate the suitability and efficacy of these hydrogels for chronic wound management applications.

Keywords: Bacterial cellulose, hydrogels, wound management, advanced dressings, wound healing

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Chimeric recombinant protein of Brucella melitensis and Immunological Evaluation for its possible use for the diagnosis of milk

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Brucellosis is a disease caused by intracellular Gram-negative bacteria, which belongs to the *Brucella* genera and is distributed worldwide. In humans, the infection is easily transmitted through the consumption of unpasteurized milk or fresh cheese. The diagnosis is based on serological methods, which are not always sensitive or specific due to cross-reactivity with other bacterial antigens. We evaluated single domain variable domain of heavy chain (VHH) antibodies against the chimeric recombinant protein BruD, which contain the epitopes Omp25, Bm26 and the Omp31, in milk artificially infected using the DotBlot assay. The antibodies VHH anti-BruD recognize the strain *B. abortus* 1119-3 up to 60 ng, so it is promising for a better diagnostic anti-brucella test

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The β-catenin/c-Myc axis in the modulation of Autophagy by Ammonia in the liver

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Autophagy is a cellular catabolic process in which cytoplasmic material is delivered to lysosomes for degradation, aids in maintaining cellular homeostasis and supplying substrates for energy generation.

Ammonia is produced from catabolism of nitrogen-containing molecules and is efficiently converted in non-toxic urea and glutamine by the healthy liver. Ammonia has recently been found to induce hepatic autophagy at low concentrations and to inhibit the autophagic process at high concentrations.

However, the mechanisms by which ammonia regulates autophagy are not completely understood.

Wnt/β-catenin signaling is emerging as a forerunner for its roles in many aspects of liver biology. This prompted us to investigate the functional association between the β-catenin/c-Myc axis and the modulation of autophagy by ammonia, in vitro and in vivo.

The results of this study unravel a role of the β-catenin-c-Myc axis in the modulation of autophagy by ammonia thus allowing the formulation of new treatment options for hyperammonemia.

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From a vision to a product: is my idea enough to succeed?

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Marine biotechnology has been advancing in the last 30-40 years, especially since the 20th century, when marine biologists started teaming up with transdisciplinary teams for large marine explorations. Indeed, marine organisms have adapted to survive and prosper in these, sometimes harsh, environmental conditions. Consequently, these organisms and their compounds are becoming of increasing interest in food, feed, cosmetics, medicine and other industries.

By addressing societal challenges, marine biotechnology can improve the economy and wellbeing of European regions and its inhabitants. Using our case study of microorganisms biodiscovery from Sečovlje saltpans to produce ingredients for the locally-sourced cosmetics products, we will answer the question – is (only) a good idea enough to succeed? These milestones will be addressed:

1. Technological bottlenecks: develop and optimize methodological protocols for halophilic/halotolerant microbial species isolation, identification, bioactive compound isolation/purification, biomass production, large-scale production requirements.
2. Logistical bottlenecks: development of supply chains, including the necessary infrastructure.
3. Value chain bottlenecks: development of products/ingredients with the customer/end-user in mind. Is there a need, willingness to pay?
4. Cost efficiency bottlenecks: are we developing solutions that can compete with the current alternatives on market?
5. Horizontal bottlenecks: Who are our allies? What is our overall strategy to succeed?

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Chemical and biochemical composition of jellyfish mucus and its potential in biotechnology application

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When stressed/during reproduction jellyfish can release large amounts of mucus, a complex matrix rich in proteins and glycoproteins. Yet, the exact composition on compound-level and function of mucus remains largely unexplored. We performed chemical analyses on mucus of four different Scyphozoan jellyfish species harvested in different European waters: *Aurelia aurita* s.l., *Periphylla periphylla*, *Rhizostoma pulmo* and *Cotylorhiza tuberculata*. We used colorimetric analysis for total protein and carbohydrate content, HPLC for amino acid and monosaccharide analysis, SDS-PAGE for protein and glycoprotein separation, FTIR, ^{13}C NMR and LC-MS/MS spectroscopy for structural and protein analysis. Our results confirmed that jellyfish mucus is a highly complex and heterogeneous biomaterial, mainly composed of proteins, mucins and carbohydrates. Absolute concentration of analytes differed between species, but their relative proportions are comparable. Half as many proteins were identified in *C. tuberculata* and *P. periphylla* mucus compared to *A. aurita* (due to lack of jellyfish protein database). More than 70 % of identified proteins and glycoproteins are involved in calcium and metal ion binding, suggesting potential mucus function. In addition, we found structural proteins, enzymes with antioxidant activity as well as self-protective proteins such as metalloproteinase, serpins and superoxide dismutase, indicating potential in cosmetic and medical application.

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Biotechnological Overproduction and Extensive Analysis of Polysaccharide-rich Fungal Biomass

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Mucoromycota filamentous fungi are considered as a powerful cell factory for production of broad spectrum of high-value metabolites, such as lipids, proteins, and polysaccharides. Targeted overproduction of desired bio-compounds achieved by the precise optimization of scaled-up fermentation processes may allow direct use of obtained biomass in various fields, including food/feed processing, pharmaceutics, or agro-industry. Chitosan, a copolymer of N-acetyl glucosamine and glucosamine, is extremely valuable product of Mucoromycota fermentation processes and these fungi are the only known natural producers of chitosan. Currently, chitosan is produced by deacetylation and multi-step extraction of shellfish-based chitin, and thus more sustainable and environmentally friendly alternatives are needed. Here, the basic characteristics of semi-industrial scale bioprocesses and methods of chemical analysis of produced fungal polysaccharides will be presented. The Biospectroscopy and Data Modeling group at NMBU has developed novel analytical techniques for chemical characterization of fungal biomass and cell-wall biopolymers. The techniques are based on vibrational (infrared/Raman) spectroscopy techniques which do not require complex chemical pretreatment of samples.

Keywords: chitosan, glucosamine, Mucoromycota, spectroscopy

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MichelIdentifying Potential Design Interventions for Heart-Lung Machines

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Heart-lung machine (HLM) overtakes the functions of the heart and lungs during cardiovascular (open-heart) surgeries. Perfusionists, trained personnel, are in charge of operating, maintaining, and calibrating HLMs. There are some risks associated with cardiovascular surgery due to mistakes made by perfusionists. This creates adverse effects for patients undergoing cardiovascular surgery, operators using the device, and hospital management. In this study, the functions and working principles of HLM, the needs and expectations of perfusionists, and the experience of perfusionists during cardiovascular surgeries are examined in order to determine the points that can be improved with design interventions in various hospitals in Turkey during 2021-2022. The primary aim of the study is to decrease the frequency of human-based errors in the usage of HLM during cardiovascular surgeries by enhancing the perfusionist's experiences. To understand the requirements and expectations of perfusionists regarding the HLMs, user interviews and surveys are carried out. To analyze how perfusionists engage with one another in a given context and pinpoint areas where perfusionists' experiences could be enhanced, user observations are utilized. The research findings are described in a way that is useful to those working on the design of medical devices, product designers, and design researchers.

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Innovative treatments for Eating Disorders

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Eating disorders (EDs) are serious mental conditions characterized by a disturbance in eating-related behaviors and correlated with distressing thoughts and emotions without a specific pharmacologic approach. Patients with anorexia nervosa (AN), bulimia nervosa (BN), and binge eating (BE), the three most common EDs, very often present comorbidity, such as anxiety, mood disorders, and personality disorders. Family studies together with candidate gene association approaches have shown that EDs are heritable. Single nucleotide polymorphisms (SNPs) in the serotonin receptor (5-HT2AR) and transporter (SLC6A4), in the dopamine receptors (DRD2, DRD4) and other genes, such as brain derived neutrophic factor (BDNF) have been identified in our cohort as potentially playing a role in the development of EDs. New horizons have been outlined in the biochemical filed: tryptophan and kynurenines were investigated in the patients' sera and their levels change considerably during EDs. Moreover, we found in AN subgroup high levels of very long chain fatty acids (VLCFAs), this could be an important key to understand the metabolism after starvation state in AN.
In conclusion genomics, metabolomics and lipidomics taken together are useful for an early diagnosis together with new therapeutic approaches.

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Omega rabbit: food for health benefit

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The human diet in Western countries has an altered ratio of n-6/n-3 fatty acids (15:1 vs 4:1), with a consequent increase in chronic, cardiovascular, and reproductive diseases. This project intends to produce a new functional food, Ωrabbit meat, which contains high levels of n-3 PUFA, feeding rabbits with diets rich in n-3. The consumption of Ωrabbit meat can reduce the n-6/n-3 ratio and have beneficial effects on human health. The project intends to improve the productive and reproductive performance, disease resistance, and welfare of the rabbit. The shelf-life of the Ωrabbit meat will be prolonged by using an innovative packaging, favoring national market and exportation. Ωrabbit meat will be produced within ΩRABBIT Consortium under its logo and regulations. This new food supply chain includes different actors (farmers, feed mills, breeders, sectioning companies, and traders) that can increase their profitability. Finally, thanks to the project, the socio-economic and health conditions of countries participating in the project can improve.

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Exosomes released under ceramide treatment carry miRNA regulating embryonic hippocampal cell differentiation

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Exosomes are one of the three primary types of extracellular vesicles, and their role in cell-to-cell communication has been well established. However, the specific function of exosomes in the differentiation of embryonic hippocampal cells remains unknown. In this study, we present evidence that ceramide can enhance the release of exosomes to propagate its differentiating effect to neighboring cells. Furthermore, we demonstrate that ceramide changes the expression of miRNA in exosomes compared to control cells, up-regulating 10 miRNAs and down-regulating 28 miRNAs. Notably, we discovered that some of the over-expressed miRNAs (mmu-let-7f-1-3p, mmu-let-7a-1-3p, mmu-let-7b-3p, mmu-let-7b-5p, mmu-miR-330-3p) regulate genes involved in embryo development and cell differentiation. Additionally, we focused our attention on the functions of mmu-let-7b-5p, as it can regulate genes involved in sphingolipid metabolism and neuronal development. To investigate the effect of exosomes in vitro, we cultured embryonic hippocampal cells in the presence of exosomes released after ceramide treatment at various concentrations. Subsequently, we demonstrated that exosomes can stimulate the formation of both neuronal and astrocytic cells. These findings are significant in understanding the role of exosomes in the hippocampus and exploring their differentiating effects to counter cognitive decline in neurodegenerative disorders or to promote delayed brain development in newborns.

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Whole-Cell Biosynthesis of Capsaicinoids by *Saccharomyces Cerevisiae*

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Chilli peppers contain compounds named capsaicinoids that are responsible for the spicy flavour, but they also possess pharmacological properties. They activate the mammalian TRPV1

(vanilloid receptor 1) heat receptor, a target for pain relief treatments, which makes capsaicinoids a key drug candidate.
Capsaicinoids consist of a vanilloid moiety and a fatty acyl chain connected by an amide bond. In the plant, they are produced from the precursors vanillylamine (derived from vanillin) and a medium chain acyl-CoA, which can vary in saturation, branching and chain length, resulting in structural variety among capsaicinoids. Moreover, capsaicinoids yield in the plant is affected by environmental and genotypical factors and is relatively low, thereby requiring large plantations for production.
An alternative production method is microbial biosynthesis, by transferring the relevant enzymatic pathways to a model production host. This could lead to production of plant-derived and novel capsaicinoids from sugar, renewable raw material or direct precursors. In this work, *Saccharomyces cerevisiae* was successfully engineered to produce nonivamide, a representative capsaicinoid, from vanillin and nonanoic acid. Challenging reactions of interest were *in vivo* transamination of vanillin to vanillylamine and the final amide-forming reactions. Expression of several heterologous enzymes, adjustment of reaction conditions and strategies for reducing by-products were addressed.

Keywords: Capsicum, lignin-derived substrates, amide, aminotransferase, N-acyltransferase, CoA-ligase

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Transaminase Immobilization via magnetic particles: Leveraging DES for Enhanced Substrate Solubility

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Our work addresses the challenge of enhancing substrate solubility for effective enzymatic transamination. We investigate the utilization of deep eutectic solvents (DES) to improve the solubility of benzyl acetone (BA) for transamination into 1-methyl-3-phenylpropylamine (MPPA) using ω-transaminase.
The exploration of DES reveals that Choline Chloride: Urea (ChCl: U) in a 50% mixture with phosphate buffer (pH 8) yields exceptional results, boosting solubility nearly fourfold compared to phosphate buffer alone. This optimized DES system demonstrates its potential to overcome solubility limitations, enhancing the efficiency of transamination reactions.
We further enhance enzyme immobilization using functionalized magnetic microparticles activated with glutaraldehyde, which increased the retained activity twofold compared to non-activated magnetic microparticles. The covalent binding of enzymes to the magnetic particles' surface improves stability and efficiency.
In our continuous transamination setup, a coil microtubing around cylindrical magnets immobilizes the enzyme. This microreactor design enables ongoing transamination of the BA in the (ChCl: U) DES + 50% phosphate buffer system, facilitating continuous bioconversion into MPPA. Our work offers a comprehensive strategy to enhance solubility, enzyme immobilization, and continuous transamination, showcasing the potential of DES-driven enzymatic transformations.

Keywords: Flow Biocatalysis, Magnetic Immobilization, Deep Eutectic Solvents, ω-transaminase

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Photosynthetic Microalgae Encapsulation Through Bio composite-Core Beads for Production of Valuable Metabolites

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Photosynthetic encapsulation of microalgae has a profound effect on the production of high value compounds. The present work involves the design and synthesis of novel hybrid alginate silica beads for encapsulation of living microalgae *Chlamydomonas sp.* for the extracellular diffusion of enzyme laccase using sol-gel technique. In this study, efficiency of hybrid beads was investigated by varied compositions of alginate, silica and polycation such as poly-L-lysine (PLL) and polydiallyldimethylammonium chloride (PDADMAC). The hybrid beads were evaluated in terms of biomass growth, encapsulation efficiency, protein leakage, photosynthetic and laccase activity. The synthesis of microalgae hybrid beads w ith a combination of Alginate +Silica/$CaCl_2$+PDADMAC has proven the best bio compatible matrix with homogeneous distribution of the silica with high mechanical strength. It yielded a Type II isotherm based on IUPAC classification and major pore size was around 18 nm. The highest specific laccase activity obtained was 16 U/L, at pH 3.5 resulting with biomass concentration of 1.765 g/L. EDS spectra showed the peaks of oxygen and silica indicating the viability of cellular components in the polycation solution. This technique entails the microalgae encapsulation in hybrid beads with potential application for valuable metabolite production in a sustainable way.

Keywords: Microalgae, Encapsulation, Sol gel process, Silica, Polycations, Laccase.

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The pump control importance of the pressure sensor assigned to the roller pumps in the Heart Lung Machine

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The role of pressure sensors in pump regulation is very important in the Heart Lung Machine. Pressure sensors continuously monitor and control the blood pressure in the device's circu-

latory system. In this way, parameters such as the speed and power of the pumps can be adjusted according to the needs of the patient. Pump regulation is vital to ensure that the patient's blood circulation continues in a stable manner and to ensure that the surgery is performed safely.

PID (Proportional-Integral-Derivative) control algorithms are a basic control strategy used in feedback control systems. These algorithms use feedback data to enable the system to approach the desired target and maintain the desired state.

The aim of this study is to observe the action of the pumps in pressure regulation in Heart Lung Machines, to understand the clinical need and then to determine the appropriate control mechanism. In order to obtain the desired response, PID controllers must be adjusted according to the system they control. This adjustment is carried out by determining the coefficients Kp, Ki and Kd in the most appropriate way according to the desired response.

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New insights in granulosa physiopathology: the role of ion channels and mitochondria biogenesis

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Granulosa cells (GCs) support the development of the oocyte from its earliest primordial follicle stage, playing an essential role in the oocyte maturation and human reproduction, providing energy and supporting steroid synthesis. Voltage-gated potassium (Kv) channels are the principal determinants of membrane potential in a variety of cell types in living organisms, however their functional roles in the reproductive system is less understood. These channels may themselves be gonadotropin-responsive, how these channels may influence GC responses to gonadotropins and their downstream functions in ovulation remains to be elucidated. Poor ovarian response (POR) to stimulation is one of the greatest challenges in assisted reproduction technology (ART). Diminished ovarian reserve (DOR) and advanced maternal age are considered the main etiologic factor for POR, but the underlying physiological mechanisms are still unknown.

Aim: In this study we intend to characterize K + voltage-dependent currents in poor responders and normo responders GCs, with the aim of increasing our understanding of the physiology of these cells and lend to future therapies.

Methods: Follicular hGCs are obtained after oocyte retrieval from twenty patients undergoing in vitro fertilization (IVF) during assisted reproductive techniques at the Centre of Reproductive Medicine, GeneraLife Umbria, Umbertide, Italy. Electrophysiological recordings include the patch clamp technique in whole-cell dialyzed configuration.

Results: Preliminary data show a predominance of a new voltage activated outward K + currents in hGCs from both groups of study and an alteration of the biophysics of these currents in POR responder patients.

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VDR gene SNP in thyroid disease

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The genetic factors and epigenetic modifications have significant contributions to auto-immune thyroiditis (AIT). It is suggested that there is an association between Vitamin D receptor (VDR) polymorphism and thyroid disease, among considered AIT. We aim to investigate the Frequency distribution of VDR FokI (rs2228570) genotypes and alleles in AIT. The VDR FokI gene SNP was investigated in 200 samples (100 - from healthy and 100 from AIT population (the Adjara, Georgia). We also investigated vitamin D levels according to CC, CT, and TT genotype carriers. The genomic DNA was extracted from the peripheral blood samples. The polymerase chain reaction was evaluated to examine the VDR FokI rs2228570 SNP polymorphism. The Frequency of the CC genotypes of VDR FokI (rs2228570) is high in AIT compared to the control group; Also the Frequency of C-allele is high in the Adjarian population. Notably, the studies revealed Vitamin D deficiency in carriers of the TT genotype within AIT, compared to the healthy population (p>.0.0001).

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Keywords: Autoimmune thyroiditis, VDR, SNP, Vitamin D.

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MeJa increased Resveratrol production in callus regenerated from the black grape variety (Boğazkere) grown in Türkiye

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The goals of the study are; in vitro regeneration, callus production and increasing resveratrol production in vitro regenerated samples of the black grape variety grown in Türkiye. Leaf and shoot segments of the indigenous black grape variety Boğazkere were used as explants for plant regeneration. Plant clones and callus were obtained in Murashige and Skoog (MS) medium supplemented with different plant growth regulators in different concentrations and combinations. In vitro callus and plant regeneration was achieved. Resveratrol quantity was increased in plant and tissue samples subjected to different concentrations of methyl jasmonate (MeJa) compared to the control. After

treatments, intracellular compounds were extracted from the samples and quantitatively analyzed using High Performance Liquid Chromatography (HPLC). The highest resveratrol concentration was provided from callus produced in MS medium supplemented with 2 BAP + 1 NAA and 100µM MeJa, with a quantity of 2.186 mg/g-FW. The greater presence of resveratrol, particularly in manipulated callus, compared to control samples highlights the significant outcome of being able to enhance its synthesis through elicitors.

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BioProcess Intensification & Optimization

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In this work, the microscale (bio)process development based on scale-up/numbering-up concept in combination with model-based design and optimization is presented. The main features of microscale systems are reflected in fluid dynamics, therefore the understanding of fundamental mechanisms involved in fluid flow characteristics at the micro scale is essential since their behavior affects the transport phenomena and microfluidic applications. Transport rates, reaction kinetics, and phase contact are represented by unique time constants that facilitate the understanding and representation of these processes through the "heat map" of characteristic times. Characteristic times are estimated based on first principles and controlled by the user to support meaningful analysis of chemical processes and provide insight or suggestions for successful design decisions. Theoretical description of transport phenomena and the kinetics at the micro scale is discussed and illustrated on the case of continuous biotransformation in a microscale bioreactor with yeast cells immobilized in a hydrogel film.

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Informations on the presence of indianscad (Decapterus Russelli Rüppell, 1830; Pisces, Carangidae), a non native species in the Butrint Lagoon (Saranda, Albania)

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On the end of the October 2022, some specimens of the non-native Lessepsian fish species (*Decapterusrusselli*) were found in the Butrin Lagoon (North-Eastern Ionian Sea, Albania). It was not recorded before the presence of this species in the coastal waters of Albania. Morphometric and meristic analyzes were performed for three individuals, with the aim of verifying their belonging to the species *Decapterusrusselli*.
For our specimens, average value of body width (BW) was 14.32% of the standard length (SL). The ratio between the average values of the standard length (SL) and the total length (TL) was 0.81,whereas the ratio between the standard length (SL) and the body height (BH) was 4.14.Our results of theanalyzes for the measurable characters,prove that body high (BH) is as 24.15% of the standard length (SL).Snout length (SnL) is as 37.75% of head length (HL), head length is as 27.68% of standard length (SL) whereas eye interval (EI),or interorbital distance,is as 28.76% of the head length (HL).

Keywords: alien (non-native) species, lagoon, morphometrics, meristics, gill rakers, branchiostegal rays, cleithrum.

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OneCarbonBioTM: Methanol-based biochemical production using microbial cell factories

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Currently, most of the chemicals and materials our society needs are produced from either fossil fuels or agricultural crops. Using fossil-fuels releases over 1 billion tons of CO_2 into our atmosphere each year. Crop-based production is less emission-intensive, but there is simply not enough farmland on the planet to support both chemical and food production at the same time. OneCarbonBioTM is a biotechnological platform developed by Acies Bio that aims to solve both issues. It replaces fossil-fuels and crops with methanol as the raw material for chemical production. Methanol is the ideal feedstock; it is a versatile, energy rich compound that can be produced using green technologies such as renewable hydrogen and CO_2 capture. It is available in large quantities worldwide, easy to transport, and store. It can be produced anywhere with access to CO_2, electricity, and water – no oil or crops required. OneCarbonBioTM utilizes specialized microbes to convert methanol into high-value products. These microbes naturally consume methanol and convert it into various compounds they require to sustain themselves and multiply. Acies Bio used the proprietary OneCarbonBioTM genome-editing toolbox to tame them into reliable cell factories that can be tuned to a large array of chemical products.

Keywords: methanol, fermentation, industrial biotechnology, carbon capture