POSTER PRESENTATION ABSTRACTS

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Preparation and Characterization of Novel Cross-linked Arginine Deiminase Aggregates for Improved Activity, Stability, and Reusability in Industrial Applications

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Arginine deiminase (ADI) has shown great potential in the industrial production of nutraceutical citrulline. However, till now ADI has not been commercialized due to its low catalytic efficiency, instability, and poor yield. Cross-linked enzyme aggregates (CLEA), a carrier-free enzyme immobilization technique has been considered a plausible strategy to circumvent these limitations. In the present study, ADI was precipitated from *Pseudomonas furukawaii* and cross-linked with glutaraldehyde to obtain novel cross-linked arginine deiminase aggregates. Both OFAT and multi-objective genetic algorithm was employed for optimizing the conditions of ADI immobilization. ADI-CLEA showed 0.30 IU/mL activity and 60% recovery using 40% ammonium sulfate, 20 mM glutaraldehyde, and 3 hours of cross-linking. The structure, size and stability of ADI-CLEA was studied using techniques like SEM, TEM, DLS, and Zeta potential. Results showed that ADI-CLEA were ultra porous, monodisperse with a particle size of 800 nm and -17 mV zeta potential. In comparison to free ADI, ADI-CLEA were thermally more stable, retained 50% activity at 60°C. ADI-CLEA also showed improved pH and tolerance to denaturants. Storage stability and reusability analysis further elucidated that ADI-CLEA were stable for two months and could be used industrially for more than seven batches for the enhanced production of nutraceutical citrulline.

Keywords: ADI, ADI-CLEA, immobilization, MOGA-NN, glutaraldehyde, citrulline

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Improving HDL functions by interaction with novel bioactive lipids

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Introduction: High-density lipoprotein (HDL), with its complex structure, prevents atherosclerosis due to its antiatherogenic properties. Improving HDL function and quality is expected to attenuate atherosclerosis and reduce CVD risk.

Methods: we isolated a novel active compound from Nannochloropsis microalgae (Lyso-DGTS), which increased the activities of the main antioxidant enzyme associated with HDL (paraoxonase 1-PON1). We aim to examine the effect of lyso-DGTS on rePON1 and HDL activities in vitro, in vivo, and ex vivo.

Results: Lyso-DGTS increased the activities of PON1 and HDL cholesterol efflux from macrophages in a dose-dependent manner and significantly increased the ability of HDL to induce nitric oxide (NO) production from endothelial cells. In an ex-vivo experiment, HDL obtained from 5 patients with plaque stenosis > 50% as determined by cardiac CT was incubated with \ without lyso-DGTS and measured for its HDL efflux ability. On average, HDL efflux significantly increased in a dose-dependent matter after incubation with lyso-DGTS. In serum obtained from apoEKO mice treated with Lyso –DGTS for 28 days, Lyso-DGTS increased the activities of PON1 and enhanced HDL cholesterol efflux.

Conclusion: Novel bioactive lipids based on Lyso-DGTS derivatives interact selectively with HDL components, altering their structure and functions. Improving HDL functions using Lyso-DGTS and its derivatives might be a novel approach for reducing atherosclerosis development and decreasing CVD risk.

Keywords: Atherosclerosis, HDL, lyso-DGTS, cholesterol efflux, nitric oxide, PON1.

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New Analogs of Temporin Modified with Unnatural Amino Acids

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Temporins are group of 10 to 14 amino acids antimicrobial peptides. They are highly active against gram-positive bacteria, as well as some bacterial strains resistant to some antibioteics.

Thus, they are promisinc alternative for combat of bacterials resistant to the antibiotics. Temporin A (FLPLIGRVLSGIL-NH2) is a highly hydrophobic peptide containig many basic amino acids in the primary structure. It is secreted by the skin of the red frog Rana temporaria. Herein, we report the synthesis, isolation and characterization of several new temporin A analogs modified with unnatural amino acids in position 13. The temporin analogs were synthesized by the Fmoc/OBut solid phase peptide synthesis. The structure and purity were established using HPLC-MS technique. The antibacterial properties of the synthesized analogs were examined and compared to those of temporin A and F and will be reviewed.

Keywords: temporins, antimicrobial peptides, unnatural amino acids, SPPS

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Design and Biological Studies on New Peptide analogues of tetrapeptide FELL

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The pain can be caused by various factors. It represents an unpleasant feeling and is associated with discomfort and disability. Daily a huge number of painkillers belong to different chemical groups are prescribed. However, the need of drugs with targeted analgesic activity still remains grate. Peptides are promising alternative of the conventional chemotherapeutics due to their small size, natural mechanism for elimination, low or lack of secondary effects, etc. FELL is a derivative peptide from human calcium binding protein spermatid 1. There is data that it is responsible for anti-inflammatory activity. Taking into account all previous studies on the tetrapeptide FELL, herein we report synthesis and biological activity of FELL structural analogs. The design of new molecules includes replacement of natural amino acids in the position 3 and 4 with their unnatural analogs. In addition, C-terminal COOH function is transformed into amide.

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New analogues of (KLAKLAK)2 Containing Unnatural Amino acids Dap and Dab

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$(KLAKLAK)_2$ is 14 amino acids peptide with both antimicrobial and antitumor properties. It is able to detect the negatively charged cancer or bacterial cells and to cause mitochondrial swelling and membrane destruction leading to cell apoptosis. Many strategies are used in medicinal chemistry in order to improve pharmacological properties of new molecules. Our previous investigations reveal that creation of bioconjugates with second pharmacophore or new molecules based on $(KLAKLAK)_2$ with changed primary structure is promising alternatives for obtaining of new molecules with better activity and pharmacodynamic. Herein, we report the synthesis, isolation and characterization of new $(KLAKLAK)_2$ analogues by replacement of natural amino acid Lys in the primary structure of peptides with unnatural analogues 2,4-diaminobutyric acid (Dab) and 2,3-diaminopropionic acid (Dap). All compounds have been obtained via solid-phase peptide synthesis, Fmoc/OBut strategy. They were fully characterized, their purity was investigated by means of HPLC and the structure was proven by MS. All compounds were tested for their antimicrobial effect against model strains bacteria *Bacillus subtilis* and *Escherichia coli* and fungi *Candida albicans*. The obtained results will be fully discussed.

Keywords: $(KLAKLAK)_2$, antibacterial properties, unnatural amino acids, Dap, Dab

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Potential of Self-assembling Peptides in the Enamel Lesion Treatment

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Dental caries is an unevenly distributed, biofilm-mediated, preventable disease with a considerable burden on economics and quality of life. The microorganisms contained in the biofilm produce acids as a result of their normal metabolism, which

in turn leads to caries lesion formation. The application of self-assembling peptides (SAPs) could stop the process of enamel lesion formation but the process of its repairing also depends on the ability to fight microbial biofilm formation. Herein, we report the study of the antimicrobial activity of several SAPs containing covalently bonded fluorine in the primary structure against the yeast strain *Candida albicans* NBIMCC 74 which is able to form biofilm on the teeth surface. The strain was selected due to the possible direct role of yeasts in the caries etiology of healthy populations. The antimicrobial assays were performed in triplicate by using the classical disc-diffusion method. The obtained results give a precondition for the future use of SAPs in dental medicine.

Keywords: Self-assembling peptides, Candida albicans, artificial enamel lesion treatment

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Temporin A and F New Analogues Modified with Unnatural Amino Acids Dap and Dab

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Microbial resistance against used in the medicinal practice antibiotics reaches pandemic proportions. This necessitates the search for new alternatives to existing antimicrobials. Such alternatives are antimicrobial peptides (AMPs). Temporins are antimicrobial peptides containing 10-14 amino acids, which are secreted from different animals like frogs and wasps. They are in general amphipathic and α-helical. Temporins have high activity against Gram-positive bacteria, as well as some bacterial strains resistant to vancomycin and methicillin. Temporin A (FLPLIGRVLSGIL-NH_2) is a highly hydrophobic and basic peptide, secreted by the skin of the red frog *Rana temporaria*. Temporin F is an analog of temporin A where Arg residue is replaced by Lys (FLPLIGKVLSGIL-NH_2). Herein, we report the synthesis of several new temporin A analogs modified with unnatural amino acids in position 7. The temporin analogs were synthesized by the Fmoc/OBut solid phase peptide synthesis (SPPS). The structure and purity were established using HPLC-MS technique and newly synthesized analogs were fully characterized. The antibacterial properties of the synthesized analogs were examined and compared to those of temporin A and F and will be reviewed.

Keywords: temporins, antimicrobial peptides, unnatural amino acids, SPPS

Acknowledgements: This study is funded by the National Program "EUROPEAN SCIENTIFIC NETWORKS" of the Ministry of Science and Education of Bulgaria, project D01-278/05.10.2020 „Drug molecule".

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Possibilities for improving sow reproductive management

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Pigs, being prolific animals, to increase individual piglet production, farm management must find solutions to increase sow productivity, as it decreases after 6-7 parturitions. Farms with large sow herds must pay close attention to the productive longevity of the sows, as breeding or procuring replacement sows is expensive and it makes economic sense to keep the sows on the farm as long as they are economically productive. To reduce non-productive days, as the average oestrous cycle of 18-22 days only repeats over 17-22 days, it is necessary to increase the productivity of sows by synchronizing oestrus, timing the farrowing date, stimulating sows to farrow at the same time and clustering farrowings . Knowledge of breeding sow behavior is essential to this critical management skill because both non-heat and heat behavior make it easier to identify when a sow is entering heat and when sows are peaking heat. To increase the productivity of sows, management measures must contribute to reducing the causes of insemination failure by not detecting the peak of heat and technologies that provide measures to stimulate heat, detect heat, perform insemination at the optimal time and control returns.

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Methods For Forecasting And Planning Production in Sows Of The Mangalita Breed

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Integrated farms for the production and traditional processing of meat from the Mangalita pig breed, must forecast and plan their sow production in such a way as to reduce their risks regarding biosecurity, constant supply of meat to the market and reduction of environmental pollution in the farm area. The research conducted looks at how to forecast and plan sow production, the environmental issues and challenges faced by the free range meat production system. Although the exploitation of sows of the Mangalița breed in the open air offers multiple benefits for the population, the increase in the consumption of meat and traditionally processed meat products has serious consequences for the areas in the vicinity of this type of exploitation. The assessment of sow availability and production

resource limits, together with the availability to apply policies, makes the forecasting and planning of Mangalitsa sow productions possible by designing new rearing and free-range systems using high-input-output scenarios raised, based on precision livestock farming, intensification of individual production for the expression of hereditary zest through nutrition control, maximizing the efficiency of meat production, minimizing the impact on the environment and improving the level of inputs and outputs or low input-low output, which has the economic result of lower efficiency of production, using cheaper local resources for nutrition, obtaining lower returns to reproduction, growth and fattening for meat and high prices for consumers of meat and meat products due to its special taste quality, being a production system based on the good reproductive capacity of sows, adaptability and resistance to outdoor exploitation conditions in traditional systems.

Keywords: sows, production, forecasting, planning

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Improving Reproductive Management in Sows Operated In Classic Production Systems

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The improvement of reproduction management involves the optimization of the utilization index of sows while waiting for artificial insemination by reducing non-productive days based on managerial solutions that improve the waiting period for insemination and implicitly individual productions by obtaining superior technological indicators per sow during a year of production. In order to reduce the number of non-productive days in the breeding sector, greater attention must be paid to the nutrition of sows while waiting for insemination, early control of pregnancy, reduction of the age at weaning of piglets to obtain a number of 2.5 farrowings/year, stimulation of the onset of puberty early in primiparous, induction and timing of oestrus in sows and induction and timing of ovulations in the first 4-5 days after piglet weaning. In order to improve the breeding methods and obtain the predicted pork productions, managerial measures are required to increase the number of farrowings per year, the prolificacy of sows and the weaning of a large number of piglets per sow per year. The methods of reducing non-productive days and improving reproductive management are based on nutritional control measures depending on the condition of sows waiting for insemination, early detection of estrus, insemination and repeated insemination at the optimal time of ovulation, constant distribution of farrowings throughout the year and supplementing with gilts to achieve the number of planned inseminations and reducing the weaning age of piglets to 21-25 days.

Keywords: production system, sows, management, reproduction

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Ways to Improve The Management during The Gestation Period of Sows

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Production management must lead to the improvement of ethological and technological management, because in the gestation phase in sows, three systems act in a coordinated manner, pre-implantation endocrine, metabolic-homeostatic-endocrine and post-implantation endocrine, and for its maintenance and development in normal conditions, they are necessary measures to ensure well-being and maintenance, by controlling the development of this physiological phase in the life of sows and using the technological spaces built at the optimal economic level. The management implemented during the gestation period contributes to the improvement of feeding methods through the best management of nutrition during the months of gestation, ensuring well-being through the control of microclimate factors and effective maintenance of the sows according to their physiological state in individual or shared stalls. Managerial measures concerning the improvement of pregnant sow maintenance systems and nutrition must contribute to avoiding hormonal imbalances that cause non-implantation of zygotes, embryonic mortality and early abortions. The system of keeping pregnant sows in individual or common stalls has major influences on reproductive indices even though the fecundity percentage is similar to 94.40% at 90 days of gestation, the best prolificacy is achieved in pregnant sows kept in individual stalls 11, 80±0.31 piglets compared to 11.40±0.22 piglets when maintained in common pens, the difference not being significant at a $p>0.05$ significance threshold. The weight of piglets at farrowing was higher in sows kept in individual stalls of 1350±0.24 grams, compared to 1294±0.30 grams in those kept in common stalls, the difference was statistically significant (t-test) at a significance threshold $p<0.05$.

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Primis, the First In VitroFertilization Albanian Water Frog-A New Hope for the Conservation of Endangered Amphibian Species

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The Albanian water frog, *Pelophylax shqipericus*, represents

an endangered to-extinction species, and known populations are currently in decline. Pollution, habitat loss, and aggressive exploitation are among the most important threats to its population loss. This work presents the first effort done in Albania to produce the first 40 individuals of *P. shqipericus* conceived through a successful *in vitro* fertilization (IVF) procedure. After the manipulation of adults, gametes were collected and fertilization was performed with a rate of success of about 27-38%, representing a comparable with those previously reported from other anuran species. High rates of fertilization were achieved in a sperm concentration of 1.0 x 10^6 sperm/ml, and usage of stage VI mature categorized oocytes. Furthermore, additional factors such as egg jelly coat, and sperm traits, were also studied to evaluate their possible effects on IVF and embryonal and larval developmental success in anurans. This successful IVF procedure resulting in *Primis* marks a significant breakthrough in the field of amphibian conservation, paving the way for future efforts to protect endangered species facing reproductive challenges.

Keywords: *Pelophylax shqipericus*, Assisted reproduction techniques, Frogs, Conservation

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Effects of Graphene Oxide and PEG-modified Graphene Oxide on Frog Heart Activity and Rat Liver Enzymes

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Graphene and its derivative graphene oxide (GO) are attractive nanomaterials with great potential for various bioapplications. However, their rapid commercialization increases the risk of potentially harmful effects on the environment and living organisms. Nanoparticles can accumulate in the mammalian liver and specifically target the mitochondria exerting direct dysfunction. Data on potential toxic effects of nanomaterials in non-mammalian organisms are scarce. Furthermore, nanoparticles' surface modifications can significantly influence their toxic effects.

This study aimed to assess the effects of GO and polyethylene glycol (PEG)-modified GO (GO-PEG) nanoparticles on activities of rat liver enzymes – mitochondrial ATPase and diamine oxidase (DAO), and on isolated hearts from the frog *Pelophylax ridibundus*.

GO caused a concentration-dependent decrease in mitochondrial ATPase activity. No rapid uncoupling effect of GO was found on intact mitochondria. Both GO and GO-PEG stimulated DAO activity and increased the force of frog heart contractions. An atrioventricular block, ventricular extrasystoles, and heart rate variability were observed.

In general, GO-PEG exhibited more pronounced effects compared to GO. This suggests the importance of nanomaterials' surface modifications for their interactions with biological systems.

Keywords: graphene oxide, mitochondrial ATPase, diamine oxidase, heart

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Determination of Transcript Abundance and mRNA Stability of pAnox1 ORFs

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This study aims to determine the abundance and stability of three different transcripts (ORF-1, ORF-3, and ORF-5) encoded by the plasmid pAnox1, isolated from *Anoxybacillus gonensis* 05S15. Actinomycin-D, a known transcription inhibitor, was used to inhibit transcription. Initially, the optimal Actinomycin D concentration was determined through incubation of the plasmid-containing isolate at 55°C in 2 ml of Degryse medium overnight, followed by application of various Actinomycin-D concentrations (ranging from 1 to 50 mg/ml) to the cells. Later, the pAnox1-carrying cells were recultured, and the next day, subcultured into fresh Degryse medium at a 1:100 ratio. Actinomycin-D at 10 µg/ml was added during the logarithmic growth phase, and incubation continued at 55°C. At 0, 15, 25, and 30 minutes of incubation, 2 ml of cell culture was centrifuged to collect cell pellets. RNA isolation was performed from the pellets obtained at each stage. Equal amounts of RNA were used to synthesize a total of 12 cDNA samples for the three transcripts, which were subsequently analyzed using qPCR. After confirming with melting curve analysis, the results indicated that the transcripts were stable up to 25 minutes, after which degradation occurred. The half-life values were calculated as 29 minutes for ORF-1, 13.64 minutes for ORF-3, and 14.87 minutes for ORF-5

Keywords: *Anoxybacillus gonensis*, pAnox1, mRNA stability, mRNA abundance

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Re-engineering *Klebsiella oxytoca* to produce homo-succinic acid

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Klebsiella oxytoca KC004 was engineered to enhance the carbon flux through the succinate-producing pathway. The evolutionary adaptation was conducted resulting in spontaneous mutations in the developed strain named KC004-TF160. In the 100 g/L glucose, KC004-TF160 produced succinate at a concentration of 84 g/L with a yield and productivity of 0.84 g/g and 0.87 g/L/h, respectively. Acetate was deteced at 15 g/L, but no other byproducts were found. Additionally, KC004-TF160 was able to produce succinate at a yield of 0.41-0.87 g/g using a variety of sugars and sugarcane molasse for a potential low cost and alternative carbon sources. All results demonstrated that the newly developed KC004-TF160 may be useful as one of the potential microbial platforms for the commercial production of succinate.

Keywords: *Klebsiella oxytoca*, metabolic engineering, evolutionary adaptation, succinic acid

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Application of Fish Industry Waste in the Removal of Mycotoxin FB1

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This research focuses on exploring the potential of sea bass and sea bream bones and scales as biological adsorbents for binding Fumonisin B_1 (FB_1), a toxic mycotoxin prevalent in corn and corn products used in poultry feed. Mycotoxins are harmful byproducts produced by molds, posing significant health risks to humans. Current mycotoxin removal methods have limitations, making the novel biosorption approach highly promising due to its selectivity, affordability, and eco-friendliness.
The study tested two types of buffers, phosphate pH 7 and citrate pH 3, along with simulated conditions of the poultry gastrointestinal tract for FB1 binding. Results showed efficient FB1 adsorption by all three adsorbents in phosphate and citrate buffers. However, lower adsorption occurred in simulated poultry digestive tract conditions, necessitating further research and improvements to enhance adsorbent performance.
This research significantly contributes to mycotoxin removal studies and sustainable fish waste management. It highlights the potential of using biological adsorbents while underscoring the importance of continuous investigation and refinement of the adsorption process. Ultimately, this work could pave the way for safer food and agricultural products and healthier environments.

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An Assessment of Pro-environmental and Green-entrepreneurship Behaviour of Biotech Students

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Biotech is considered one of the most promising sectors to support the sustainability mission under climate change, whereas teaching and promoting green practices in Biotech education is a necessary action. The universities can do more, particularly by teaching future graduates to take action on sustainability. Under the Erasmus+ project 2021-1-RO01-KA220-HED-000032162 "*Green education for green Biotech Enterprise*", a mixed EU consortium (Greece, Italy, Romania, Spain) performed an assessment of pro-environmental and green-entrepreneurship behaviour of students enrolled in higher education in their countries. In the study, environmental literacy and environmental self-awareness were considered factors influencing pro-environmental intention. Based on 213 respondents aged 18 to 25 years, the results confirmed the initial hypothesis. Perceived environmental literacy exhibited positive appreciation; however, knowledge about legal action strategies had a very low positive score, which means that respondents were generally undecided when it came to asserting their level of knowledge regarding this particular topic. The investigated population manifested an increased environmental awareness and recognised the importance of environmental quality and the existence of environmental issues while declaring a high intention to perform environmentally friendly actions. The study also showed that students expressed a positive intention towards starting a green business.

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Comparative analysis of primary mural and cumulus granulosa cells under long term culture

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The aim was to perform comparative analysis of morphological and phenotypical characteristics of primary human mural and cumulus granulosa cells (MGCs and CGCs).
MGCs were received by the centrifugation of the follicular fluid. CGCs were isolated from the cumulus-oocyte-complex (hyaluronidase treatment). Cultivation was performed during 10 days. Phenotypical (HSD17B1, IGFBP5, COL3A1, RYR-

2) and morphological characteristics were determined on the 10th day of cultivation. Fluorescence microscopy was performed using an Olympus BX61. The results were analyzed with Student's t-test (Excel software).
The cultures were characterized by the presence of round cells, sail-shaped, stellate and spindle-shaped cellular elements. Cell proliferation was significantly higher in the CGCs compared with MGCs cultures throughout the observation period. The relative number of cells expressing HSD17B1, IGFBP5 and Col3A proteins in MGCs was higher in 2.4-, 1.3 and 2.7-times comparative with CGCs on the 10^{th} day of cultivation. CGCs had a high level of RYR2 expression, while MGCs were negative.
These results can be used to develop new reproductive biotechnologies for the application of granulosa cells as an experimental system for in vitro functional studies in the field of toxicology.
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Keywords: cultivation, phenotype, granulosa cells.

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Development of a model for predicting lipase extraction and activity in natural deep eutectic solvents

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In recent years, there has been a surge of interest in environmentally friendly extraction, which has led to the development of cleaner, more sustainable and efficient extraction techniques. One such innovative approach is the use of natural deep eutectic solvents (NADES) for downstream processing.
The use of an aqueous two-phase system based on NADES was investigated as a potential extraction tool for lipase from a liquid formulation. Choline chloride served as a hydrogen bond acceptor, while various hydrogen bond donors were evaluated for their efficiency. A total of 40 NADES were prepared and characterized (pH, density, polarity, viscosity), and the σ-profile for each NADES was determined using the COSMOtherm program. A series of batch extractions were performed to evaluate the extraction efficiency and enzyme activity for each NADES prepared. Based on σ-profiles, a predictive model for enzyme activity and extraction efficiency in different NADES was developed using the Statistica program package. Optimal NADES were proposed based on the developed models, and the results were validated using a series of independent experiments. To intensify the process, the extraction was performed in a microextractor, resulting in efficiency of E=96.47±0.872% and remaining enzyme activity of 99.20±0.64% at a residence time of 0.5 min.

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Dairy and non-dairy beverages with increased total phenolic content and antioxidant activity

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The interest of consumers in the development of enriched dairy and non-dairy products with phenolic compounds due to their possible health benefits, is increasing. Furthermore, the international market of functional foods is rising and represents one of the most attractive areas of innovation in the food sector. In the present study, the development of dairy and non-dairy beverages with increased total phenolic content (TPC) and antioxidant activity (AA) was evaluated, using fruits (either juices or pulp). The main physicochemical characteristics, total phenolic content, antioxidant activity, and viability of starters were monitored during the production and storage of beverages. The use of fruits had no significant effect on milk acidification rate and the main physicochemical characteristics of dairy beverages, affecting however those of non-dairy beverages. Significant was the effect on the color of dairy and non-dairy beverages. The addition of fruits increased significantly the TPC and AA of dairy beverages and especially non-dairy beverages. Starter and probiotic cultures retained high viable counts during storage revealing the potential beneficial effect of fruits. The results show that fruits have the perspective to be used in fermented beverage production to optimize the benefits of functional products with high phenolic compound intake.

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Effect of the Hypericum perforatum extract on the growth of probiotic lactic acid bacterial strains

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The extracts of aerial parts of *Hypericum perforatum* in different solvents have shown a wide spectrum of antibacterial activity against different pathogens, both Gram-positive *Staphylococcus aureus*, *Staphylococcus epidermidis* and Gram-negative *Salmonella* sp., *Shigella dysenteriae*, *Yersinia enterocolitica*, *Escherichia coli*, *Pseudomonas aeruginosa* and other. Scientific publications have presented data for established anti-Helicobacter activity against standard and clinical isolates of *Helicobacter pylori*.
The twelve LAB strains used in the present study were isolated from the oral microbiome and defined as strains with probiotic potential. These strains show well expressed antimicrobial activity against different Gram-positive and Gram-negative test-pathogens and antagonistic activities against the common

oral pathogens *Streptococcus mutans* and *Candida albicans*.
The effect of a dry water-alcoholic extract of *H. perforatum* on the growth of the twelve probiotic LAB strains was investigated. Five of the strains were found to show no growth inhibition and two other strains showed a slight retardation in growth. Five of the tested strains were sensitively affected by the extract of *H. perforatum*.
These results are important for determining the effect of the studied extract on probiotic strains from the gastrointestinal microbiome.

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Antagonistic interactions of lactic acid bacterial strains from human oral microbiome against orally associated pathogens

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Many microbial species can colonize the oral cavity, become pathogenic and cause common oral diseases. Lactic acid bacteria (LAB) are important part of the gastrointestinal microbiota and the oral cavity in particular. Studying LAB interactions with various pathogens is important as mechanisms to limit the spread and eliminate them. The use of probiotic LAB strains against oral infections by pathogenic species such as *Streptococcus mutans*, *Candida albicans* is of serious research interest and an important alternative for the prevention and treatment of such infections.
The aim of our study was to assess the antagonistic activity of oral LAB strains against two common oral pathogens *S. mutans* and *C. albicans* through different *in vitro* methods.
All studied strains showed well expressed antagonistic activity, where four of them inhibited the growth of *S. mutans* by 3–5 logs. Most of the strains showed growth inhibition activity against *C. albicans* by up to 2 logs. All tested LAB strains possess co-aggregation property with *S. mutans* and most of them showed higher co-aggregation with *C. albicans* which may exert an important host defense mechanism against infection development. The *in vitro* study showed specificity in self-biofilm formation and high anti-biofilm activity against *S. mutans* and *C. albicans* by most of the strains, above 79% and 50%, respectively.

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Identification and Characterization Of Lactic Acid Bacteria Isolated From Human Breast Milk and Infant Feces

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Since the beginning of the 21st century, studies on the human breast milk and infant fecal microbiome become increasingly worldwide. In our study the target species were lactic acid bacteria (LAB) and bifidobacteria which allow for the establishment of balanced gut microbiota of infants. The aim of the investigation was to isolate and identify LAB strains from breast milk of healthy Bulgarian women and their healthy newborns for further analysis to estimate their potential as probiotics. Samples (brest milk and infant feces) from 12 tandems (mother and baby) were analyzed. The presence of rod-shaped bacterial species was found in 75% of the examined breast milk samples and in 100% of the feces samples. The species identification was determined by classical methods and MALDI-TOF. The isolates were identified as *L. rhamnosus*, *L. fermentum*, *L. paracasei* and *L. reuteri*. In the examined samples, the predominant species were *L. rhamnosus* and *L. fermentum*. Geographic location and dietary affect human milk microbiota profiles. Thus, native probiotic strains might be more suitable for Bulgarian women than strains from other locations.

Acknowledgements: This work was supported by OP SESG, Grant number BG05M2OP001-1.002-0012 and National Science Fund, Grant КП-06-Н61/9, Bulgaria.

Keywords: breast milk, infant feces, microbiota

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Antibacterial activity of new isolated lactic acid bacterial strains against phytopathogenic bacteria

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Lactic acid bacterial strains can produce antimicrobial compounds such as organic acids (lactic acid, acetic acid), bacteriocins, and other metabolites, preventing the proliferation of different pathogens and food spoilage microorganisms. This study evaluated the efficacy of lactic acid bacteria (LAB) isolated from naturally fermented vegetable products against the phytopathogenic bacteria *Erwinia amylovora*, *Clavibacter michiganense* subsp. michiganense and *Xantomonas vesicatoria*. Five strains were isolated from naturally fermented vegetable products and identified to species as *L. curvatus* - Z3P4, *L. plantarum* - Z7P3, *L. sakei* - Z12P1, *L. brevis* - Z17P2, *L. plantarum* -T4P3. The species so defined are a natural part of the microbiota of similar food products. Cell free supernatants (CFS) from all five LAB isolates ware tested in vitro for antibacterial activity by the disc diffusion method. All five LAB were shown over 50% to 100% inhibition effects against test phytobacteria *Clavibacter michiganense* subsp. *michiganense*. Anti-

bacterial activity against *Xantomonas vesicatoria* was detected at four of LAB strains and two of studied strain were expressed antibacterial activity against *Erwinia amylovora*.
Established activity of the newly isolated LAB strains against phytopathogenic bacteria is a reasonable prerequisite for their designation as biocontrol agents in agro- and food technologies.

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Role of the KCa3.1 modulation in glioblastoma therapy

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Glioblastomas (GBs) are the most common primary brain tumor, and one of the most malignant and aggressive forms. It involves multiple alterations in protein kinase and ion channel activity, among them intermediate conductance calcium-activated potassium (KCa3.1) currents are observed in many aspects of the glioblastoma biology. We investigated molecular and cellular events of ionizing radiation (IR) on glioblastoma U251 models. By using molecular, cellular, and electrophysiological approaches, we characterized (i) blockers from *Euscorpius italicus*, which so far it represents an unexplored source of toxins; (ii) small molecules, specifically LY294002. As a 1proof of concept, we have realized silver nanoparticles conjugated with a toxin. Results suggested that KCa3.1 are involved in radiosensitizing processes and that modulators and blockers represent a next tool to optimized IR treatments.

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Biological Activities of Lactic Acid Bacteria Isolated from Traditional Fermented Foods

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Lactic acid bacteria (LAB) play a critical role in food production and health maintenance. There is an increasing interest in these species to reveal the many possible health benefits associated with them. The actions of LAB are species and strain specific, and depend on the amount of bacteria available in the gastrointestinal tract.
Central to their activities are the bioactive components they produce, including short-chain fatty acids, antimicrobial and antiviral peptides and vitamins, which play integral role in modulating inflammation, enriching nutrient absorption and fortifying the immune system. In our research we have studied the probiotic potential of several LAB as a complex of their bioactivities isolated from different food sources as a probiotics. The autoagregation and potential for biofilm development properties have been studied as part of the study of their potential as probiotics. Determination of their antibiotic and antiviral potential showed overall weak activity. Although tested strains do not synthesize bacteriocins or similar compounds some of them showed inhibition properties against *E. coli*, S*almonella sp.*, *Listeria innocua* and *S. aureus*. Antiviral activities is determined against Herpes simplex virus type 1 and type 2 in MDBK cell culture. Weak activity against viral replication have been observed against both models.

Keywords: Lactic Acid Bacteria, Probiotics, Antibiotics, Antiviral

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Pregnancy diagnosis in dairy donkey: experimental hypotheses to develop an immunological test

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Nowadays the growing use of donkey for milk production requires a concomitant improvement in the reproductive management technique. The measurement of pregnancy-associated glycoproteins (PAGs) concentrations in the maternal blood is used as a biochemical marker of pregnancy in various ruminant species. The discovery of the expression of new members of PAGs in the pig and in the mare show that the production of these glycoproteins is not exclusive of the species with synepitheliochorial placenta. We aimed to evaluate the possibility to identify PAGs in the serum of donkeys utilizing several different PAG antisera usually used as a pregnancy test in other species. Serum from females at different stages of pregnancy and from prepubertal males as control were utilized. PAGs analysis was performed with a radioimmunoassay technique. The PAG gene expression on term placentas was also investigated using molecular biology. Among the different antibody tested, that against buffalo PAG seems to better recognize PAGs in donkey. The application of endpoint PCR has allowed to obtain a preliminary result on the presence of Equus Asinus PAG gene expression in placental tissue. These findings encourage further investigations to develop a reliable pregnancy test in this species.
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Porungo cheese whey: a new substrate exploited to enzyme and GOS production

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The use of agro-industrial residues in bioprocess engineering encourages the obtention of bioproducts, such as enzymes and prebiotics. This research investigated the GOS production by β-galactosidase immobilized on chitosan-genipin supports from porungo chese whey - a residue pioneered for exploitation by our research group. The enzyme was produced from *Kluyveromyces marxianus* using porungo cheese medium (30°C and 200 rpm), with a total enzymatic activity of 517.90 U. Prior to GOS production, two immobilization strategies were tested. The chitosan-genipin allowed higher stability at slightly acidic pH (6.0-6.5), while the alginate support showed higher relative activities at slightly more basic pH (6.5-7.0). The GOS production was carried out using the enzyme concentration of 100 U/mL and three different porungo cheese whey concentration (200-400 g/L of lactose) at temperature of 40 °C. The highest yield of GOS3 (15.24 %) was obtained at a substrate concentration of 300 g/L, representing a 45.21% increase compared to that obtained from 200 g/L, and a lactose conversion 6,31% higher than that obtained from 400 g/L (47.16%). This research can contribute to a more sustainable production of biomolecules with high economic and technological interest in the food, pharmaceutical and agricultural industries.

Keywords: agro-industrial residues; galactooligosaccharides; immobilized enzyme; whey; β-galactosidase.

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Valorization of agrifood residues, as a raw material in the paper industry

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Agro-industrial vegetable waste is ideally suited for valorization in packaging materials due to its high lignocellulose content and high amount of phenolic compounds. These materials may offer competitive benefits to paper companies as specialized packaging materials, allowing them to use a low-cost feedstock to make a bespoke, high-quality output. In our contribution, we focus on the production residues of various plant streams (e.g. growing and processing of onions, olives and pomegranates), to demonstrate how such diverse agro-industrial bio-waste materials could be fully exploited even before their reformulation. Both onion skins and olive leaves demonstrated high antioxidant activity as well as the ability to recover high yields of bioactive chemicals (roughly 100 mg of quercetin or oleuropein per g of dry extract). The amount of antocyanins in pomegranate peel, on the other hand, was modest (approximately 0.3 mg of antocyanins per g of dry extract). Each of the three plant residue sources yielded a different type of paper. Olive skins and olive leaves were employed as separate feedstocks, but pomegranate peel had to be mixed along with cellulose. The produced papers have good technological properties as well as an unusual texture and look, suggesting that they could be used to make specialty papers.

Keywords: Crop residues, vegetable residues, bioactive compounds, paper production

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Study of IFI16 protein interaction with G4 prone DNA sequences and its influence on p53 transcription activity

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The IFI16 protein interactions with DNA are important in many biological processes such as regulation of replication, transcription and translation. The binding properties of IFI16 to G4 prone sequences and the effect of this bond on reporter gene transcription, induced by the well-known tumor suppressor p53, were studied in a yeast isogenic system. The G4 prone DNA sequences, with the p53 *PUMA* binding site (derived from the *PUMA* gene promoter), were located upstream of a minimal gene promoter *LUC1*. The KSHV sequence derived from the G4 herpesvirus KSHV and its adenine-substituted mutants KSHV-1NO and KSHV-3NO were analyzed by CD spectroscopy. The binding of the IFI16 protein to these sequences was studied by EMSA. The CD spectroscopy results showed the formation of parallel G4 of KSHV and KSHV-1NO sequences in potassium ion-containing buffer. *In vitro* competitive

EMSA demonstrated IFI16 protein interactions with CD retrieved G4 formation sequences, without significant binding to PUMA or KSHV-3NO oligonucleotides. *In vivo* experiments showed a repression of *LUC1* transcription due to stabilization of KSHV by IFI16 protein and an increase of *LUC1* transcription due to p53 guidance to its binding sites by IFI16 protein in the case of absence of any IFI16 binding site.

Keywords: IFI16, p53, G-quarduplex, DNA-protein interactions, protein-protein interactions, transcription activity, Yeasts one-hybrid isogenic systém, EMSA, CD spectroscopy

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Exploring the Evolutionary Role of G-Quadruplexes and Examining Their Potential Impact on Viral Pathogenesis

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Hepatitis B virus (HBV), a dangerous human pathogenic virus, coexists with Hepatitis Delta virus (HDV), a highly unusual RNA satellite virus that relies on the presence of HBV to induce infection. The significance of G-quadruplexes (G4s) in virus genomes is becoming more evident. G4s are formed from guanine-rich sequences and were found in various genomes including the human genome and viruses. With G4s recognized as potential therapeutic targets in virology, we analyzed G-quadruplex-forming sequences (PQS) in 232 modern and ancient HBV genomes and 474 HDV genomes using G4Hunter predictions to pinpoint G4s within accessible viral genomes. Interestingly, the density of PQS motifs is lower in ancient HBV genomes than in their modern counterparts. This modern frequency is very close to the PQS frequency of the human genome. This indicates that the PQS content in HBV increased over time to become closer to the PQS frequency in the human genome. Contrary to the HBV, the HDV PQS frequency is more than four times higher suggesting the important role of G4s in this genome. These findings suggest an important role for the PQS in HDV genomes. Moreover, the high propensity of the PQS could serve as a good therapeutical target for deregulated HDV replication.

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Bovine colostrum supplementation modulates cytokines, vascular and antioxidant gene expression in the gut of rabbit

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The study aimed to assess the effects of bovine colostrum (BC) diet supplementation on the inflammatory and antioxidant gene expression in the rabbit intestinal tract. Thirty female NZW rabbits were divided into three groups receiving commercial feed (control group) and the same diet supplemented with 2.5% and 5% BC. From their litters, 15 young rabbits were selected for each group that received the same diet as their mothers from weaning (35 d) to slaughter (90 d). At slaughter, tissue samples from the jejunum, cecum, colon, and meseraic lymph node were collected for RNA extraction. Gene expression was evaluated via qRT-PCR. The results indicate that 5% of BC supplementation determines the upregulation of IL-8 in the jejunum and facilitates the migration of immune cells. BC promotes the expression of genes that reduce inflammatory responses (TGF-B) and regulate the gut-vascular barrier permeability (CTNNb1). Finally, the 5% BC supplementation enhances antioxidant activity in the cecum and colon. These results suggest that BC can influence the inflammatory and antioxidant response of the intestinal tract of rabbits, although further research is required.

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Comparative Analysis of Neurotransmitters in Ovarian Follicular Fluid: A Study on Polycystic Ovary (PCO) Patients Undergoing IVF Treatment

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This study aimed to investigate the presence and levels of neurotransmitters in the ovarian follicular fluid of polycystic ovary (PCO) patients compared to a control group. The study included 20 patients undergoing in vitro fertilization (IVF) treatment, with 10 individuals diagnosed with PCO and 10 in the control group. Liquid chromatography mass spectrometry was employed to analyze the follicular fluid, revealing the identification of 333 metabolites.

The statistical analysis was conducted using Metabo analyst, focusing on metabolites of interest. Among these, L-glutamic acid, L-aspartic acid, and dopamine were identified as potential neurotransmitters, but no significant differences were observed between the two groups (p-values of 0.3, 0.8, and 0.7, respectively). Additionally, neurotransmitters such as adrenaline and noradrenaline were not detected in the analyzed metabolites.

Based on these findings, it can be inferred that there is no discernible difference within this specific family of metabolites in the follicular fluid of PCO patients compared to non-PCO patients. Further research and investigations are warranted to comprehensively understand the role of neurotransmitters in PCO and their potential implications in the context of IVF tre-

atments.

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Antibacterial effect of an active absorbent pad on rabbit meat in modified atmosphere packaging during shelf life: preliminary results

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The study aimed to evaluate the efficacy of an active absorbent pad in limiting microbial growth during the shelf-life of fresh rabbit meat in modified atmosphere packaging (MAP). The experiment was conducted on 30 portions of regions of the thigh muscles, each packed in MAP (70% O2, 30% CO2), one containing the conventional pad (control group) and the other containing the active pad (PAD group). The analyses, performed at 1, 7, 14, and 21 days of refrigerated storage, concerned total viable count (TVC), *Staphylococcus* spp., and *E. coli*. In general, there were no differences between the two types of packaging for all microbiological parameters investigated. Regarding the trend over time, at the end of shelf-life, a significant increase ($p < 0.05$) was observed for TVC and *Staphylococcus* spp. but not for *E. coli*. Interestingly, TVC decreased after 7 days in the PAD group, but not in the control group. This decrease needs to be further investigated to define the effectiveness of the MAP+ active pad combination on rabbit meat microbiological profile.

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The impact of hypoxia and inflammation on primary human granulosa cells

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Premature ovarian insufficiency (POI) is a pathological condition that is associated with significant impairment of women's reproductive, physical, and mental health. Ovarian granulosa cells represent supporting somatic cells whose physical contact with the oocyte is essential for its growth, maturation, and ovulation, and, thus, their dysfunction as a result of adverse environmental events or factors may contribute to the pathogenesis and development of POI.

Here, we sought to elucidate the proliferative, apoptotic, and functional responses of primary human granulosa cells to both hypoxia and inflammatory conditions. The results showed that exposure to inflammatory culture conditions significantly increased the rates of early- and late-stage apoptosis, inhibited proliferation, and decreased the secretion of estradiol and progesterone by granulosa cells. However, while hypoxia had no effect on the rates of early-stage apoptosis, cell proliferation, or estradiol production in these cells, it substantially increased the percentage of cells in late-stage apoptosis and inhibited their estradiol secretion.

These findings suggest that chronic inflammation- and oxygen deprivation-induced alterations in the local environment may lead to disturbances in the normal growth, viability, and functions of human granulosa cells, which may contribute to follicle dysfunction and impaired ovarian activity.

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Analysis of Biochemical Compounds in Cherry Laurel Pulp

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This study aimed to identify chemical composition of Georgian endemic Cherry Laurel (wild and cultivated forms) using modern physico- chemical methods. Improvement of the technologies for obtaining bioactive compounds, based on applying the principles of environmentally friendly circular economy. Four phenolic compounds were identified from the pulp of cultivated (Prunus Lusitanica) and wild (Laurocerasus officinalis) forms. Phenolic carboxylic acids and both, aglycone and their glycoside of anthocyanins were separated and identified on the HPLC, UPLC-PDA-MS analytical and preparate column. For the determination of total monomeric anthocyanins AOAC official method 2005. To determine antioxidant activity we used DPPH method. The Folin- Ciocalteu reagent for quantifying total phenols. We discovered, that wild ripe fruit that grows at the highest point above the sea level is the richest in anthocyanins and has twice as high antioxidant activity then of the cultivated ones. In conclusion, can be said that cherry laurel is the promising crop for making medications, containing bioactive compounds, which by evidence are effective to prevent and treat several medical conditions, such as CVD, DM, cancer etc. Especially, the wild forms of this plant, which is not distinguished by taste qualities, is the best raw material for this purposes.

Keywords: cherry laurel, anthocyanins, bioactive compounds, antioxidant activity.

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Study of bioactive compounds of the technological process of wine making from red grapes

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The use of biologically active additives in the food and pharmaceutical industries has increased especially recently due to their various protective actions. In this regard, the polyphenolic compounds contained in red wine are particularly noteworthy. It is also interesting to study the bioactive compounds in wine production residues.

Determination of biologically active compounds: anthocyanins, leucoanthocyanins, catechins, total phenols and antioxidant activity of red grape (chkhaveri) residue spread in Western Georgia using modern instrumental research methods: HPLC; UPLC-MS-PDA. For determination of antioxidant activity (using stable radical of 2.2-diphenyl-1-picryl hydrazyl) DPPH method.

Chkhaveri fermented chacha, fermented pods, fermented defatted pods contains: total phenols-1198.24;1578.38;2507.13, Catechins-321.35;641.74;316.90 leucoanthocyanins-551.61; 338.30;208.58mg/100g (d.w.), antioxidant activity 50% inhibition 0.1mMDPPH by 0.421; 0.154;0.288mg sample (respectively).

The residue of Chkhaveri wine is a raw material rich in biologically active compounds and with high antioxidant capacity, for the preparation of both drugs and biologically active supplements.

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Keywords: grape (chkhaveri) residue, leucoanthocyanins, catechins, antioxidant activity

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Bioproduction of aromatic compounds from C1-C2 compounds using metabolic engineered methylotroph

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The production of chemicals from CO_2-derived raw materials will greatly contribute to improving the CO_2 balance in the environment. C1 or C2 compounds derived form CO_2 (e.g. methanol and ethanol) can be converted to synthesize linear hydrocarbons with various chain lengths, but to synthesize aromatic compounds is difficult via C1 chemical process.

In this research, we aim to develop a technology to produce aromatic compounds (styrene) at a high yield using methanol and ethanol by bioprocesse. We use the methylotrophic bacterium *Methylorubrum extorquens* PA1 as a production host. First, we introduced exogenous genes involved in styrene production into wild-type *M. extorquens* PA1 to produce styrene from methanol. Next, we constructed knock-out strains in which competing metabolic pathways were disrupted to improve styrene yield. Wild-type *M. extorquens* is known to oxidize approximately 80% of the methanol it consumes to CO_2. We constructed four formate dehydrogenases (FDHs) in *M. extorquens* PA1 (Δ4). In the Δ4 strain, formate accumulated in the methanol medium, indicates that carbon loss through FDHs-mediated oxidation of formate to CO_2 is prevented. In addition, the Δ4 strain consumed more ethanol than the wild-type in ethanol minimal medium, resulting in improved cell growth rate and cell density.

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Encapsulation of EPS-producing Limosilactobacillus fermentum strains for improved functional properties

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Lactic acid bacteria (LAB) *Limosilactobacillus fermentum* strains MC1, isolated from breast milk, and D12, isolated from smoked cheese, are producers of exopolysaccharides (EPSs). Numerous bacterial species, fungi, and certain algae are capable of producing EPSs. However, EPSs produced by LAB are of great interest due to their GRAS status and the possibility of their use as probiotics. EPSs have been shown not only to improve the taste and extend the shelf life of fermented foods, but also to have antioxidant, antibacterial, antiviral, anticoagulant, and antitumor effects, and to lower cholesterol levels. To further enhance these properties, MC1 and D12 strains were encapsulated. The *L. fermentum* strains were nanocapsulated using layer-by-layer method, and the positive role of the freeze-dried nanocapsules on the survival of the strains under simulated gastrointestinal tract (GIT) conditions and after storage was demonstrated. Moreover, the same strains were microencapsulated using alginate as matrix, with and without the addition of the prebiotic substrates fructooligosaccharides and galactooligosaccharides, and it was shown that microencapsulation of potential probiotic strains improved survival in simulated GIT conditions and during storage.

Keywords: *Limosilactobacillus fermentum*, exopolysaccharides, nanoencapsulation, microencapsulation, functional properties

[Abstract: 112] DOI: 10.2478/ebtj-2023-0019

Biocompatibility and metabolomic application of novel teflon-based Solid Phase Microextraction (SPME) devices in vitro.

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Solid Phase Microextraction is a promising technique for metabolomic studies. The currently available coatings can be used for either non-volatile or volatile compounds. However, Teflon-based SPME devices could be used for the simultaneous extraction of both types of compounds. In the course of this study, Teflon-based SPME devices were tested for cell culture-based metabolomic studies of non-volatile compounds.

The SPME devices were prepared in house at Middle East Technical University. B16F10 and LL2 cell lines were used for the studies. After 24, 48, 72 and 96 hours of cell culturing the extractions were conducted using new devices. After the last extraction, MTT, BrdU and apoptosis assays were performed. The extracts obtained with SPME were subjected to untargeted metabolomics analysis with LC-HRMS.

The Teflon-based SPME devices did not influence cellular viability and growth. Moreover, metabolomic information, such as the number and range of compounds, obtained with Teflon-based SPME were comparable with commercially available coatings. Therefore, these enhanced SPME devices can be successfully used for the analysis of non-volatile compounds, and their use for volatile compound analysis is currently being explored.

The project is financed by the National Center for Research and Development (NCBR), project number POLTUR4/MicroIVIVE/5/2021.

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Diabrotica v. virgifera Resists the Effects of Entomotoxic Fungal Protease Inhibitors in Food

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The western corn rootworm (*Diabrotica virgifera virgifera*, Coleoptera:Chrysomelidae) is a major invasive maize pest that is difficult to control. Protease inhibitors of higher fungi are exceptionally stable proteins, making them suitable candidates for climate-smart pest management. We confirmed their resistance to proteolytic digestion and inhibition of the cysteine catalytic type of proteolytic activities in gut extracts of larvae and adults of *D.v.virgifera*. However, bioassays showed no effects on neonatal mortality or stunting, no effects on adult mortality, and no effects on egg hatching. Microscopic analysis of the peritrophic matrix and apical surface of midguts showed the similarity between larvae of *D.v.virgifera* and the coleopteran chrysomelid *Leptinotarsa decemlineata*, which has previously been shown to be sensitive to these inhibitors. This suggests that resistance to the protease inhibitors is likely due to effective adaptation of digestive enzyme expression to dietary protease inhibitors. Nevertheless, we continue to investigate the environmentally friendly entomotoxic proteins from higher fungi as potential biopesticides to control the major invasive agricultural pests. We acknowledge funding from ARIS, Slovenia (J4-2543, P4-0432 and P1-0184) and NKFIH, Hungary (134356 SNN20).

[Abstract: 114] DOI: 10.2478/ebtj-2023-0019

Application of Teflon-HLB SPME fibers to analyse volatile metabolites in LL2 and B16F10 cancer cells.

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Diverse types of Solid Phase Microextraction (SPME) assays are often used in in vitro metabolomic studies focused on development of new cytostatic drugs or investigation of cancer pathogenesis. This approach allows multiple analysis from a single sample gaining more insight in phenotype response triggered by investigated factor.

Recently developed coating for SPME fibers that consists of HLB adsorbent particles embedded in the Teflon matrix was proved to be suitable for both thermal and solvent desorption, enabling the analysis of a wide range of analytes. In the present study this novel HLB-Teflon coated SPME fibers were used to assess the effect of Combretastatin A4 on metabolome of LL2 and B16F10 cancer cell lines. For the chosen cellular metabolites detection limits of (HLB)SPME-GC-MS analysis ranged from 2.3 to 12.4 ppb, precision ranged 1.4 to 18.3 %RSD, and accuracy was 98-120%. For both cell lines the exo- and endo-metabolome were investigated revealing altogether 36 volatile compounds at significantly different levels in cells compared to respective reference samples.

Presented study was supported by the National Center for Research and Development (NCBR) under the program MicroIVIVE, project number POLTUR4/MicroIVIVE/5/2021 and by the Scientific and Technological Research Council of Turkey,

grant number TUBİTAK 120N352.

[Abstract: 115] DOI: 10.2478/ebtj-2023-0019

Inducing biological and nutritional stress in the cultivation of carotenogenic yeast and autotrophic microalgae

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This study was focused on the effect of biological and nutritional stress induced on various autotrophic microalgae and carotenogenic yeast. Nutritional stress was ensured by adding selected waste oils (coffee and frying oil) to the media, and biological stress was achieved by cultivating microorganisms within one medium in so-called co-cultivations.
The monitored properties were the production of biomass and metabolites and the production of lipids and lipophilic substances. All types of these stresses tended to lead to an increase in production of both biomass and monitored metabolites. The studied metabolites were carotenoids, chlorophylls, coenzyme Q, sterols, and fatty acids.
The studied yeast was *Rhodotorula kratochvilovae* and *Rhodotorula toruloides*. Representatives of microalgae were *Desmodesmus acutus*, *Scenedesmus obliquus*, and *Desmodesmus communis*.
The addition of waste oils in co-cultivations with yeast *R.kratochvilovae* tended to have the best production of both biomass and metabolites compared to other tested yeast. The best co-cultivation experiments with added waste oil showed up to be *R. kratochvilovae* with *Desmodesmus communis* and *R. kratochvilovae* with *Scenedesmus obliquus*. In microalgae experiments, it was found that nutritional stress in the form of added glycerol to the medium had an inhibitory effect on the growth and metabolism of microalgae.

[Abstract: 116] DOI: 10.2478/ebtj-2023-0019

The influence of waste substrates on mixotrophic growth of microalgae

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Microalgae are known mainly as a large group of photoautotrophic organisms producing a number of important metabolites such as carotenoids, chlorophylls, lipids, and many others. Their mixotrophic potential was investigated by adding an alternative organic carbon source to the medium.
The chosen carbon sources were two types of waste oils from the food industry. The coffee oil was obtained from spent coffee grounds. The waste frying oil is a low quality product of fast food industry produced in large quantities.
The aim of this work was to test the stress effect of waste frying oil and coffee oil on the growth of algae and the production of metabolites, as well as the possible utilization of the waste oils. The results of this experiment served as a precursor for further experiments on microalgae with waste substrates.
The growth and production characteristics of microalgae *Desmodesmus armatus*, *Chlamydomonas reindhartii*, *Scenedesmus acutus* and the *cyanobacteria Anabaena torulosa* were tested. The results showed enhanced production of chlorophylls by the strain *Desmodesmus armatus* and increased lipid accumulation in the strain *Chlamydomonas reindhartii.*

[Abstract: 117] DOI: 10.2478/ebtj-2023-0019

The study of the physical-chemical characteristics of field honey, common in Western Georgia

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Honey has been used since ancient times due to its high nutritional value and healing properties. the chemical composition and the melissopalynological analysis determined naturalness and origin of honey, including field honey.
The present work deals with the study of the chemical characteristics of field honey collected in Western Georgia,using modern instrumental research methods-HPLC RI, UV/Vis, Conductivity Detectors, UPLC PDA,MS (Waters).
The quantitative and qualitative content of carbohydrates, total phenols, flavonoids, phenolic acids and cations in twenty field honey samples.common in various regions of Western Georgia,as well as the characteristics provided by the Regulations on Honey Codex Alimentarius (Codex Standard for Honey, 2001): determination of moisture, of electrical conductivity, of ash content, pH and free acidity, of diastase activity, of proline, pollen content and antioxidant activity.
As a result of the research,it was found that the water content in field honey ranges from 16,9% to 22.4%,respectively,the dry matter is 76.6%-85.1%, free acidity is 10.9–30.0 meq/kg , active acidity -0,2146-1,287,electrical conductivity-0,2146-1,287 mS/cm, total phenols-171,60-705,54mg/kg,phenolic acids-147,90–253,54 mg/kg,antioxidant activity at 50% inhibition of the DPPH radical per mg sample-60,68–225,65, proline-976,31–990,83mg/kg, diastatic activity-up to 8,5–20.

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[Abstract: 118] DOI: 10.2478/ebtj-2023-0019

Qualitative study of biologically active compounds of fruits and leaves of Elaeagnus umbellata Thunb. using UPLC-PDA, MS method

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There is an increasing demand for natural compounds with various activities and their food sources. Most frequently, there are used preparations derived from such plants, which, unlike synthetic analogues, are cheaper and have no side effects. In this regard, Elaeagnus Umbellata Thunb., rich in biologically active compounds, is of great interest and is being studied in many countries of the world.

The object of the research was a qualitative study of biologically active compounds in the fruits and leaves of Elaeagnus Umbellata Thunb., introduced in Western Georgia, using the UPLC-PDA, MS method.

In the course of the study of flavonol glycosides in the fruits and leaves of Elaeagnus umbellata Thunb., using the UPLC-PDA, MS method, a total of 19 phenolic compounds have been identified, including 16 substances found in the fruits: Quercetin-O-(pentosyl) hexoside-O–rhamnoside, Isorhamnetin-O-glucuronide derivative, Quercetin-O-(-pentosyl)hexoside, Sinapic acid-O-pentosyl(hexoside), Kaempferol-O-(coumaroyl)hexoside, Quercetin-O-(pentosyl) hexoside-O–hexoside, Diosmetin-8-C-hexoside-C-hexoside, Sinapic acid-O-hexoside, Dihydrokaempferol-O-hexoside, Quercetin-O-hexoside, Kaempferol-O-dihexoside-O-rhamnoside, Diosmetin-O-dihexoside, 2-Methylaconitate derivative, Kaempferol-O-dihexoside, Kaempferol-O-hexoside, Isorhamnetin-O-hexoside. There are 7 substances in the leaves, among which 4 substances (Quercetin-O-(pentosyl)hexoside, Sinapic acid-O-pentosyl(hexoside), Kaempferol-O-(coumaroyl)hexoside, Kaempferol-O-hexoside) are similar with the fruit, and 3 ones are different: bis-HHDP-O-glucose, Saccharide, Isorhamnetin-O-pentosyl(hexoside).

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[Abstract: 119] DOI: 10.2478/ebtj-2023-0019

3D printing and post-printing modifications of individualized polycaprolactone scaffolds for bone tissue regeneration

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The treatment of critical-sized bone defects often requires grafts. Three-dimensional printing is a suitable method for manufacturing grafts with precise patient-specific customization of scaffold geometry. Polycaprolactone (PCL) is a widely used suturing material - hydrophobic semicrystalline bioresorbable polyester. Its physicochemical properties allow the production of spatially organized porous structures that have a stable architecture. In addition, the osteoinductive PCL can be used as a carrier for mesenchymal stem cells (MSCs). Thus, personalized PCL-MSC implants are potent in promoting effective bone regeneration. The aim of our study is to produce PCL scaffolds loaded with MSCs for the treatment of critical-sized bone defects in a mouse model.

Using 3D Fused Filament Fabrication, PCL structures have been created following two factors: the total print size and the pore dimensions of the matrix. Prints without cells were implanted in mice, and the resulting bone regeneration was analyzed by microcomputed tomography.

As MSCs showed poor adherence to printed PCL, the scaffolds were subjected to post-printing modifications, including incubation in hydroxyapatite solution and in 3M NaOH. Scaffolds were then loaded with MSCs, cultured, and analyzed using confocal microscopy.

The results showed osteoinductivity of the PCL scaffolds implanted in mice's calvaria as well as better adhesion of MSCs to the scaffolds after NaOH post-printing modification.

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Isolation, Characterization Of Pseudomonas aeruginosa Phages As An Anti-Biofilm Agent, And Their Inhibitory Effects On Biofilm Formation

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Pseudomonas aeruginosa is a pathogenic microorganism known for adhering to various surfaces to form biofilms, which are significant in medical, industrial commercial systems and diverse industries. However, due to its natural structure and the presence of inactive cells within the biofilms, this microorganism is resistant to antibiotics and various chemical agents, making it difficult to eliminate the biofilm structure. This study contributes essential insights into alternative antimicrobial therapies, highlighting a potential path forward in the ongoing battle against antibiotic-resistant biofilms. Our research focuses on the isolation and characterization of *Pseudomonas aeruginosa*-specific phages and investigates their potential inhibitory effects and phage-antibiotic combinations on biofilm formation of *Pseudomonas aeruginosa* strains. This approach aims to illuminate the potential for bacteriophages as a viable alternative in combating biofilm-associated economic losses and untreatable diseases caused by *Pseudomonas aeruginosa* both worldwide and domestically. The understanding of bacteriophage's role in biofilm formation and eradication provides a promising alternative method in anti-biofilm strategies and

presents an opportunity for innovative treatment protocols.

Keywords: bacteriophage, biofilm, *Pseudomonas aeruginosa*, transmission electron microscopy

[Abstract: 121] DOI: 10.2478/ebtj-2023-0019

Gas-liquid chromatography of Prunus vodka

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The alcoholic fermentation of Prunus cerasifera Ehrh juice and other products accured spontaneously over a period of approximately 15-20 days. The resulting tkemali distillate was subjected to gas-liquid chromatography analysis using a TRACE™ 1310 GC from Thermo Scientific. Chromatography was conducted on a SGE BPX5 Capillary GC Column.
Several methods were used to obtain Prunus cerasifera Ehrh vodka and to establish the relationship between methanol and pectin content in the fermenting mass. Interestingly, removing pectin from the juice did not lead to a decrease in the methanol content in the obtained vodka. On the contrary, it was slightly higher (0.085%) than in Prunus juice (0.053%) and in vodka obtained from Prunus juice and pulp (0.053%). Research in this direction should be continued, although the content of other components was significantly reduced, and propanol was not detected, while in other samples, it is up to 0.1%. There was also a significant difference in isobutanol content, with 0.05% and 0.9% and 1.9% in juice and pulp, respectively. The same was true for isoamyl derivatives. In vodka obtained from the mass purified from pectin, it was practically absent, while in other samples, it was almost more than 2% in total.

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Effect of extracellular vesicles secreted by bladder cancer cells on non-malignant uroepithelial cells, an in vitro study

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Extracellular vesicles (EVs) are membrane-derived particles secreted by cells into the extracellular space. There are many types of EVs differing in size and origin. EVs are responsible for transporting proteins, lipids and nucleic acids to specific target cells. Cancer cells secrete significantly more EVs than normal cells do. The transfer of substances produced by cancer cells through EVs to the environment affects many stages of cancer progression.

The aim of the study was to evaluate the effect of EVs secreted by bladder cancer cells on non-malignant urothelial epithelial cells. EVs were purified from the conditioned media of bladder cancer cells (HT-1376) and then evaluated for their effect on non-malignant uroepithelial cells (SV-HUC-1). EVs concentration and size distribution were assessed using the TRPS technique. Immunophenotype of obtained EVs was checked with nanoflow Cytometry. The effects of EVs on cell viability (MTT assay), proliferation rate (xCELLigence), cell cycle (flow cytometry) and epithelial-mesenchymal transition (EMT) (n-cadherin, e-cadherin and vimentin fluorescent staining) were examined.
EVs secreted by bladder cancer cells affect the properties of non-malignant cells.

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Metabolic engineering of Corynebacterium glutamicum for hydroxybenzoic acid production

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Hydroxybenzoic acids (HBAs), including 4-HBA, 3-HBA, and 2-HBA, are useful platform chemicals for commodity materials and fine chemicals production. We employed metabolic engineering to enhance the production of these HBAs in *Corynebacterium glutamicum* as a host. We enhanced the shikimate pathway and eliminated genes associated with HBA degradation, particularly phenol 2-monooxygenase encoded by *cg2966*. Increased titers of 3-HBA and 4-HBA were achieved via selection of suitable promoters for 3-hydroxybenzoate synthase and chorismate pyruvate lyase. Efficient production of 2-HBA was enabled by maintaining a balanced expression of isochorismate synthase and isochorismate pyruvate lyase. Consequently, strains KSD5-tacM1-H and KSD5-J2-PE showed high titer of 19.2 g/L of 3-HBA and 12.9 g/L of 2-HBA, respectively, using 1 L jar fermenter containing 80 g/L of glucose. These engineered strain has significant potential for production of other valuable products derived from chorismate.

Keywords: *Corynebacterium glutamicum*, 2-hydroxybenzoic acid, 3-hydroxybenzoic acid, Metabolic engineering

[Abstract: 124] DOI: 10.2478/ebtj-2023-0019

m-Nitrobenzoate synthesis by Escherichia coli with N-oxygenase

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Nitroaromatic compounds are widely used in industry, however, chemical synthesis of them has some problems such as sa-

fety concerns and low selectivity. An alternative way that can overcome them is enzymatic synthesis. In this study, we utilized AurF: *p*-aminobenzoate *N*-oxygenase, which synthesizes *p*-nitrobenzoate from *p*-aminobenzoate by oxidizing the amino group. We previously achieved *p*-nitrobenzoate production from glucose by *E.coli* introduced with AurF. To expand the range of nitroaromatic compounds that can be synthesized by AurF, our next aim was set to produce *m*-nitrobenzoate. First, we investigated the capacity of AurF to convert *m*-aminobenzoate to *m*-nitrobenzoate. Our result demonstrated that the conversion rate is 6 % and it is approximately 1/13 of that of *p*-aminobenzoate. Next, we introduced mutation around the active site of AurF to enhance its activity to *m*-aminobenzoate. AurFY93F, T167I showed the highest conversion rate, 80 %, which is equal to the conversion rate of *p*-aminobenzoate by wild-type AurF. In addition, we achieved *m*-nitrobenzoate production from glucose by introducing AurFY93F, T167I and PctV, which synthesizes *m*-aminobenzoate from 3-dehydroshikimate.

Keywords: nonnatural nitroaromatic compound, *E.coli*, *N*-oxygenase, mutation

[Abstract: 125] DOI: 10.2478/ebtj-2023-0019

High yield bioproduction from glucose and xylose in "PMPE" E.coli strains

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Bio-based chemical production using microbes is a promising strategy for a sustainable society. Therefore, the production of various compounds in microbial hosts is studied around the world. One of major challenges during this process is the low yield of target compounds due to using most of supplied substrates for cell growth. Our study aims high-yield production of 1,2-propanediol and resveratrol in engineered *Escherichia coli* while avoiding this problem by dividing metabolic pathways according to "PMPE". "Parallel Metabolic Pathway Engineering(PMPE)" is a metabolic engineering strategy for effective sugar utilization by separating production and cell growth. In this work, in order to produce target chemicals efficiently, genes related to pathways from glycolysis to TCA cycle in *E.coli* were disrupted according to each biosynthetic pathway. In addition, we introduced exogenous xylose assimilation pathways, weimberg pathway and dahms pathway from *C.crescentus*, to recover the cell growth of 1,2-propanediol and resveratrol producing strains respectively. After cultivation of these resultant PMPE strains, we achieved the aerobic production of both compounds from glucose and xylose. Rational metabolic engineering approaches including disruptions of competing pathways contributed to improvement of each compound productivity.

Keywords: Parallel Metabolic Pathway Engineering, Metabolic Engineering, *Escherichia coli*

[Abstract: 126] DOI: 10.2478/ebtj-2023-0019

Immobilized Enzymatic Reactors on Monolith backbone for rapid Plasmid Linearization

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The manipulation of plasmid DNA (pDNA) plays a pivotal role in different applications of molecular biology and genetic engineering. For example, linearized pDNA is essential tool in *in vitro* transcription reaction for messenger ribonucleic acid (mRNA) production. Immobilized enzyme reactors (IMERs) stand as innovative biotechnological constructs, seamlessly merging the catalytic proficiency of enzymes with the advantages of solid support matrices. Monolithic supports, characterized by their highly interconnected channels, highly accessible surface area and efficient mass transfer properties, provide an ideal backbone for enzyme immobilization. Opting for immobilized enzymes over enzyme solutions offers notable benefits such as improved stability, the potential to operate within a continuous system over extended durations, reusability of the enzyme, as well as reduced production costs and product purification steps.

We immobilized EcoRI restriction enzyme on monolithic supports and studied the restriction of model pDNA (6.7 kbp). The degree of linearization was analysed by chromatographic separation of pDNA isoforms, using CIMac pDNA analytical columns. We reported that required contact time for linearization is about 10-15 minutes and the IMERs reusability goes up to at least one month. The functionality of IMER linearization product was finally tested by *in vitro* transcription reaction mRNA production.

Keywords: IMER, restriction enzymes, monoliths, mRNA

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A preliminary in vitro investigation of anticholinesterase activity of Salvia fruticosa (sage) extracts indigenous to Cyprus

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Alzheimer's disease (AD) is characterized by memory deficits and impaired cognitive function. A deficiency in levels of the neurotransmitter acetylcholine (ACh) is observed in the brains of AD patients, therefore, the inhibition of acetylcholinesterase (AChE), the enzyme that hydrolyses ACh, is a treatment option. Commercially available drugs with AChE inhibitory activity possess side effects. Consequently, a need for utilization of alternative anticholinesterase compounds leads to the investi-

gation on plants. According to research findings, sage extracts possess diverse biological activities. In the present study, various sage extracts were quantified for phenolic compounds and assessed for acetylcholinesterase inhibitory activity in vitro. The methanolic extract contained the highest amount of phenolics, followed by the ethanolic extract. All extracts inhibited acetylcholinesterase in a dose-dependent manner, while the increased anticholinesterase activity of the methanolic (IC_{50} = 21 μg/mL) and ethanolic (IC_{50} = 28 μg/mL) extract was positively correlated with their phenolic content. Furthermore, the extracts inhibited acetylcholinesterase in a mixed (competitive/noncompetitive) manner since the K_m value of acetylcholinesterase increased in their presence, while the V_{max} value decreased. The results of the present study constitute *Salvia fruticosa* a valuable herb, which could be used as a source of bioactive compounds.

Keywords: acetylcholinesterase, inhibition, sage

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Osteogenic Differentiation Of Mesenchymal Stem Cells Cultured On Bone Implant Surfaces Is Enhanced By Vitamins

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The combination of tissue engineering and cell therapy represents a promising approach for bone defect healing. The bone-regenerating potential of mesenchymal stem cells (MSC) is well known, which makes these cells a preferable candidate for bone reconstruction strategies. For successful tissue-engineered bone repair and regeneration, it is essential to promote osteogenic differentiation of MSC as early and effectively as possible. So, the aim of our study was to follow the effects of vitamins on osteogenic differentiation of primary MSC. Our results confirm the need for a personalized approach, as osteogenic differentiation of cells from different patients is affected differently by the vitamins studied. While the differentiation of three of the four primary cultures is positively affected by vitamin D and downregulated by vitamin K, the fourth patient's MSC showed just the opposite effect: upregulation by vitamin K and downregulation by vitamin D. When osteogenesis was induced in MSC cultured on PVC cell culture plates or bone implant surfaces (β-TCP granules and TI-6AL-4V alloy), we also found specific effects. Namely Vit E and K enhanced osteogenesis in all MSC lines cultured on β-TCP, while Vit D induced the highest AP activity in MSC cultured on TI-6AL-4V alloy in comparison to other Vits studied.

[Abstract: 129] DOI: 10.2478/ebtj-2023-0019

Does the type of mesenchymal stromal cell culture media influence the properties of the secreted extracellular vesicles?

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Mesenchymal Stem/Stromal cells (MSCs) are finding increasing application in medicine. Recent studies indicate that tissue regeneration induced by the administration of MSCs cells is due to their trophic properties, including secreted extracellular vesicles (EVs).

The aim of this study was to compare the influence of media types for MSC culture and purification on the properties of obtained AD-MSC-EVs.

Adipose tissue was enzymatically digested, and Stromal Vascular Fraction cells were cultured in two GMP-grade media. Conditioned medium was collected from cultured AD-MSCs from the third passage and centrifuged to remove cell debris and apoptotic bodies. The supernatants were concentrated by centrifugation using a 10KDa ultrafilter and purified by Size Exclusion Chromatography. The size and concentration of obtained AD-MSC-EVs were analysed using Tunable Resistive Pulse Sensing. Additionally, MSC-specific surface markers on AD-MSC-EVs were investigated using nanoflow cytometry.

The results show that EVs can be successfully obtained from both media. A difference in the number of isolated AD-MSC-EVs between tested media was observed. A significantly larger size of AD-MSC-EVs was observed for unpurified EVs. Significant differences were found in MSCs-specific surface markers between EVs generated with tested media, besides lack thereof on the cellular level. This indicates novel significant therapeutic-influencing properties.

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Melatonin Affects Osteogenesis in Mesenchymal Stem Cells Cultured on Polystyrene Surface and Ti-6AL-4V Alloy

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Melatonin is a hormone that primarily controls the circadian rhythm of the body and stimulates osteogenesis in bones. Mesenchymal stem cells (MSCs) are responsible for bone reconstitution in cases of osteoporosis, bone fractures, osteoarthritis, and bone defects. Differentiation, proliferation, cell efficiency, and anti-apoptotic effects of MSCs treated in vitro with melatonin are shown to be boosted. The aim of this study is to compa-

re melatonin effects on osteogenic differentiation of bone marrow MSCs cultured on polystyrene plates and on Ti-6AL-4V alloy, a material widely used for bone implants.
Our results showed that melatonin in different doses does not affect MSCs proliferation, but alkaline phosphatase (ALP) activity in osteogenically differentiated MSCs was enhanced. However, ALP RT-PCR data concerning the melatonin effect on osteogenesis of MSCs cultured on polystyrene or Ti-6AL-4V alloy were quite controversial. While in four out of seven primary MSC lines cultured on lab plastic we recorded upregulation of ALP, the same lines cultured on Ti-6AL-4V alloy showed upregulation in three out of seven, and only in one of the MSC lines upregulation was observed in both culturing conditions. In conclusion, melatonin effects on osteogenesis are dependent not only on the cell source but also on the surface and conditions of culturing.

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Proangiogenic Properties of Rat Bladder Acellular Matrix Seeded with Adipose-Derived Mesenchymal Stem/Stromal Cells

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One of the main problems of tissue engineering is insufficient angiogenesis of reconstructed tissues. Mesenchymal stem cells (MSCs) and Extracellular Matrix (ECM) play a crucial role in this process and can promote endogenous angiogenesis via microenvironmental modulation.
The aim of the study was to evaluate potential proangiogenic properties of rat Bladder Acellular Matrix (BAM) seeded with Adipose-Derived Mesenchymal Stem/Stromal Cells (AD-MSCs).
Urinary bladders were obtained from Wistar rats and subjected to a decellularization process combining physical, biological, and chemical methods. BAM acellularity was analyzed by histological staining. The presence of residual DNA was analyzed using DAPI staining and molecular analysis. BAM cytotoxicity against 3T3 cell line and AD-MSCs in vitro was analyzed using MTT assay. Lyophilized matrices were seeded with AD-MSCs from the third passage, cultured for 7 days and analyzed for expression of proangiogenic growth factors, e.g. Vascular Endothelial Growth Factor (VEGF). Cell viability and distribution on the scaffold were analyzed by H&E and Live/Dead staining. Analysis confirmed the absence of cell nuclei and high efficiency of DNA removal (residual DNA content < 50ng/mg dry tissue weight) for decellularized bladders, high viability of AD-MSCs seeded on acellular scaffold and expression of proangiogenic growth factors.

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Application of industrial wastes in biotechnology of carotenogenic yeasts

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This research is focused on cultivation of selected carotenogenic yeasts by using industrial waste substrates. Carotenogenic yeasts are able to produce many valuable metabolites with use of many industrial sectors, included pharmaceutical and food industries. Within this experiment, it was necessary to map the growtf of these microorganisms on various components of these waste substrates, such as selected alcohols, sugars, organic acids etc. Subsequently, the yield coefficients and amounts of produced metabolites were compared, within the different strains and used source of nutrients. The studied metabolites include carotenoid pigments, ubiquinone, ergosterol, microbial lipids and β-glucans. These metabolites contained in biomas, were analyzed by various laboratory techniques, included gas chromatography (GC) and high performance liquid chromatography (HPLC). The industrial wastes used in this research were for example stills from th destillery industry, or waste glycerol originating from the production of biofuels. The results of this work can provide valuable informations with application in industrial biotechnology of carotenogenic yeasts and reduce their economic demandingness and at the time provide aid with ecological disposal of selected industrial wastes.

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Enhancing shelf-life prediction of food supplements: a novel kinetic model for thermo-oxidative stability under storage conditions

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Shelf-life is defined as the length of time a product may be stored without becoming unsuitable for use or consumption. The labels currently used on food and beverage products provide an expected shelf-life based on kinetic model for predicting thermo-oxidative stability. Accelerated oxidation method and simple kinetic model for predicting a shelf-life as accurate as possible is advisable to improve the safety, reliability, and sustainability of the food supply, reducing food waste. In this study we developed a kinetic model based on Arrhenius equation to estimate the degradation through time of a bioactive compound, such as resveratrol contained in food supplement, after the exposure to different storage conditions. HPLC analysis were performed to evaluate resveratrol degradation. Results showed a non-linear degradation of resveratrol after the expo-

sure to higher temperature through the same time, suggesting a necessity to develop a new kinetic model to predict the shelf-life of foods and supplements after accelerated oxidation tests.

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Study of bioactive compounds of the technological process of wine making from red grapes

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The use of biologically active additives in the food and pharmaceutical industries has increased especially recently due to their various protective actions. In this regard, the polyphenolic compounds contained in red wine are particularly noteworthy. It is also interesting to study the bioactive compounds in wine production residues.

Determination of biologically active compounds: anthocyanins, leucoanthocyanins, catechins, total phenols and antioxidant activity of red grape (chkhaveri) residue spread in Western Georgia using modern instrumental research methods: HPLC; UPLC-MS-PDA. For determination of antioxidant activity (using stable radical of 2.2-diphenyl-1-picryl hydrazyl) DPPH method.

Chkhaveri fermented chacha, fermented pods, fermented defatted pods contains: total phenols-1198.24;1578.38;2507.13, Catechins-321.35;641.74;316.90 leucoanthocyanins-551.61; 338.30;208.58mg/100g (d.w.), antioxidant activity 50% inhibition 0.1mMDPPH by 0.421; 0.154;0.288mg sample (respectively).

The residue of Chkhaveri wine is a raw material rich in biologically active compounds and with high antioxidant capacity, for the preparation of both drugs and biologically active supplements.

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Keywords: grape (chkhaveri) residue, leucoanthocyanins, catechins, antioxidant activity

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The influence of different types of chemical and physical stres on microalgae

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Nowadays, a lot of attention is being paid to the biotechnology industry. Within this industry, are with the help of microorganisms produced various useful substances, such as chlorophyll and carotenoids, which are important antioxidant substances, but also fatty acids, sterols and ubiquinone. This poster is focused on the cultivation of selected strains of microalgae of the genus Scenedesmus and Desmodesmus. Different types of alcohol were applied to the selected strains as stress conditions, with the aim of increasing the production of intracellular metabolites. Further, fermentor cultivation was carried out, in which a combination of two different alcohols was applied. Subsequently, cultivation using a flat- panel photobioreactor was also carried out, in which light with different wavelengths was used. Several chromatographic techniques were used for the analysis and the results of the analyzes are presented in the experimental part.

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Pre-treatments upgrade the enzyma

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Isolation of Secondary Metabolites from Marine-derived Bacteria and Evaluation of their Inhibitory Activity on α-Synuclein Aggregation

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In the ongoing search for more effective drugs of natural origin, marine microorganisms have emerged as a goldmine yielding secondary metabolites with potent biological activities.

The most common histopathological finding in Parkinson's disease, the second most frequent neurodegenerative disease after Alzheimer's disease, is the inclusions of aggregated α-synuclein (α-syn), an abundant neuronal protein. Aggregated forms of α-syn are particularly toxic to neuronal cells and contribute the most to the pathogenicity of the disease. Consequently, inhibiting the formation of α-syn neurotoxic oligomers/aggregates could restrict the disease progression.

In the project AlphaSyn, we have screened a panel of marine-derived bacterial extracts to discover agents that can remove aberrant α-syn assemblies. The most active extracts were fractionated, allowing for the isolation of their secondary metabolites and evaluation of their bioactivity.

Acknowledgements: This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation under the call RESEARCH – CREATE – INNOVATE (2nd cycle) (project

AlphaSyn/T2EΔK-02813).

Keywords: marine-derived bacteria, Parkinson's disease, α-synuclein

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Fucoidans and Alginates from Brown Algae Growing Wild or Cultivated Following an Integrated Multitrophic Aquaculture Approach

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Marine algal polysaccharides have been proven versatile agents for various applications in the biomedical and food sectors, as well as in cosmeceutics and nutraceutics, due to their diversified chemical structures and functionalities which, in turn, attenuate their biological and physicochemical properties.
In the framework of the project IMTA, the main aim is the establishment of an integrated multitrophic aquaculture for the production of fucoidans and alginates from brown algae. To this end, protocols for the isolation of fucoidans and alginates were developed, optimized and applied on brown algae of the genus *Cystoseira*, both collected from wild populations and aquacultured in the facilities of Kefalonia Fisheries following an integrated multitrophic aquaculture approach. The isolated polysaccharides were structurally characterized with NMR and FT-IR, as well as for their molecular weight, total sugar content and monosaccharides' composition, sulfate content and thermal properties.

Acknowledgements: This research has been co-financed by the European Maritime and Fisheries Fund of the European Union and Greek national funds through the Operational Programme for Fisheries and Sea 2014-2020 under the call INNOVATION IN AQUACULTURE (project IMTA / MIS 5074605).

Keywords: brown algae, integrated multitrophic aquaculture, fucoidans, alginates

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Long-Term Psychophysical Effects of Covid-19 in Pregnancy

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During the Covid-19 pandemic, numerous women contracted the virus while being pregnant, leading to intricate circumstances during this delicate period of life. The resultant isolation from family and friends, reduced health check-ups, and symptoms have posed significant challenges for these women. Additionally, in many cases, cesarean deliveries were performed, and mothers were unable to breastfeed, which further impacted the mother-child relationship. However, the long-term consequences of these experiences remain poorly researched from both psychological and physiological perspectives. The aim of this study was to enroll postpartum women at a specific time after pregnancy to assess their psychophysical well-being. The participants were contacted through email to complete a clinical questionnaire, a psychological assessment, schedule a psychological interview, and provide a blood sample. The blood sample was utilized to evaluate the protein expression of the vitamin D receptor and sphingomyelinase, molecules implicated in the symptoms of Covid-19. From a psychological point of view, preliminary findings showed the presence of anxious memories regarding the childbirth and the following weeks after the childbirth, as well as the current presence of anxious symptoms in the relationship with the child. Vitamin D receptor and sphingomyelinase were expressed at high levels, indicating a good inflammatory reaction.

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Comparison of vital parameters of pigs receiving isoflurane and sevoflurane anesthesia during the surgical operations

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Since February 2014 pigs have been used in surgical operations for training purposes at the Center of Advanced Simulation and Education (CASE) housed at Acibadem University. They undergo robotic surgery, minimal invasive general surgery, gynecology, urology and lung surgeries in operations that last an average of 6 hours. Two different anesthetics are used in the operations; isoflurane and sevoflurane. The aim of the study was to compare the vital parameters of these pigs receiving isoflurane and sevoflurane anesthesia.
White Yorkshire breed pigs used in the study. Mean arterial pressure (MAP), PCO_2, PO_2 heart rate and body temperature were monitored during the surgeries. Vital parameters at the three time points (beginning, midst, and at the end of experiment) are considered for statistical analyses.
MAP was found to be decreased in the sevoflurane group

($p<0.001$) than isoflurane group. During the first half of experiment, PCO_2 displayed a greater decrease in isofluran group ($p<0.05$), while groups were equalized by the end of the procedure. PO_2 was comparable in both groups during the first half, however it increased dramatically during second half in sevoflurane group ($p<0.05$). There was no difference between the groups in terms of heart rate and body temperature.

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Enhancing Compound Stability Using Natural Deep Eutectic Solvents: A Stabilization Study

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Over the past decade, deep eutectic solvents (DES) have gained prominence for their potential to improve the efficiency and sustainability of diverse processes. Boasting advantages such as low volatility, non-flammability, and minimal toxicity, these solvents are synthesized from readily available natural resources, foregoing the need for traditional solvents. Their remarkable trait lies in their exceptional adaptability, allowing tailored solvent design to meet specific industrial demands.

DES, originating from natural sources, can replicate environments suitable for various biomolecules. In both dehydrated and aqueous states, they not only facilitate the dissolution of diverse biomolecules but also stabilize commercially significant natural compounds. Notable examples include DNA, bioactive substances, and proteins, by induce specific molecular conformations.

This research delves into DES's ability to stabilize a range of biomolecules:

Anthocyanins: Valuable plant metabolites with strong antioxidant properties.

Hydrolytic and oxidoreductive enzymes: Investigations were conducted on stabilizing purified lipases and enzymes sourced from orange peel and various dehydrogenases.

Nicotinamide Adenine Dinucleotide Coenzyme (NAD): Vital for over 500 enzymatic reactions governing key biological processes.

The study showcases how deep eutectic solvents effectively stabilize these biomolecules, offering a versatile platform for enhancing compound stability in various applications.

Keywords: Biomolecule stabilization, Deep eutectic solvents, Natural origin, Sustainability, Tailored solvent design

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Increased level of Vasoactive Intestinal Peptide (VIP) in follicular fluid of patients with polycystic ovary syndrome during IVF

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Polycystic ovary syndrome (PCOS) is a common multifactorial and polygenic disorder of the endocrine system, affecting between 5 and 10% of women of reproductive age with a still unknown etiology. Follicular fluid (FF) represents a *milieu* necessary to normal development of follicles rich in metabolites, hormones and neurotransmitters but in some instance of PCOS the composition can be different. Vasoactive intestinal peptide (VIP) is an endogenous neuropeptide involved in follicular atresia, granulosa cell physiology and steroidogenesis and in some aspects of gonadotropin independent phase. We found in FF recovery from IVF procedure an increase of frequency of samples with higher VIP concentration (>150 picograms/ml) in PCOS (n=10, 40%) with respect to the healthy (n=10, 0%) groups (Fisher's test; p=0.08). This data indicates the presence of a subpopulation in PCOS with higher VIP concentration values with respect to control group. An inversely proportional correlation was found between the VIP and plasma FSH concentration (p=0.05 and a correlation index of -0.43). The relationship between deregulated VIP and FSH levels in PCOS pathophysiology is also discussed.

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Expression of NanoLuc Luciferase in Campylobacter jejuni as a mean to study biofilm formation

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Campylobacter jejuni is the leading cause of bacterial gastroenteritis worldwide, and biofilm formation plays a key role in its survival on food manufacturing surfaces. These biofilms represent the main risk for food chain contamination. Thus, understanding how *Campylobacter* forms biofilm and how it resists treatment will facilitate the development of novel intervention strategies. However, there is a critical need to develop a rapid, specific, and reproducible method for detecting and quantifying *Campylobacter* in biofilms, considering it can survive in a viable but non-culturable form under adverse environmental conditions. To address this challenge, we have constructed plasmid pMW10-nLuc for the inducible expression of NanoLuc luciferase in *Campylobacter*. By optimizing several parameters, we successfully transformed the plasmid into *Campylobacter*. This new construct is being used to reveal intricate behaviours associated with *Campylobacter* biofilm formation and growth, which will improve our understanding of the mechanisms involved in biofilm development.

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Keywords: *Campylobacter jejuni*, NanoLuc luciferase, bioluminescence, bacteria quantification, biofilm

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Understanding of Aspergillus ochraceus' physiology - a key to biotechnological production of proteases

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Fungal extracellular proteases (especially from *Aspergillus* genus) are widely used in biotechnology, however, selection of optimal conditions for an efficient protease production still remains a time-consuming step. In this research, we tried to understand how fermentation conditions affect diversity of proteolytic activities and protease production for *Aspergillus ochraceus* L-1.

For submerged fermentation, media containing various carbon and nitrogen sources were used. Collagenolytic, caseinolytic, fibrinolytic and elastinolytic activities were measured spectrophotometrically. Total DNA and mRNA were isolated and sequenced. The genome was annotated with AUGUSTUS. Putative extracellular proteases were predicted with HMMER and SignalP 6.0.

Combination of glycerol and glucose was found to be the optimal carbon source for production of proteases with specific activities. The highest non-specific activity was observed in the sucrose-containing medium, when glycerol as the only carbon source in the medium completely depressed protease production. Maximal collagenolytic and fibrinolytic activities were observed when cultivating with fish meal hydrolysate and bovine collagen. Highest general and elastinolytic activities were detected in the medium with elastin and $NaNO_3$.

Genome mining suggested the presence of a wide range of serine, metallo- and aspartic peptidases. The RNA-sequencing will be used to evaluate expression level of predicted proteases.

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Inhibition of renal cell death by blocking Fas-mediated apoptotic signal protects from acute kidney injury

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Acute Kidney Injury (AKI) is a sudden and rapid decline in kidney function. Severe AKI can result in irreversible loss of kidney cells and nephrons, necessitating kidney replacement therapy. While antioxidants like edaravone showed potential for clinical use, it was deemed limited due to its systemic side effects.

In this study, we tested Fas-blocking peptide (FBP) that inhibits Fas-mediated cell death in AKI. Two-time 12mg/kg FBP was administered in an AKI model 30minutes before ischemic injury and 12hours after the injury. Renal cortex damage induced Fas-gene expression 5times greater than that from normal kidney. However, FBP-treated mice showed significantly reduced lower levels of Fas expression. Histopathology confirmed less dilated tubular cell structures and 38% reduced cell death, improved serum glomerular filtration rate (creatinine, BUN), an indicator of renal function protection. Moreover, FBP-treated mice showed less impaired electron transfer chain activity in kidneys, leading to reduced reactive oxygen species production from the damaged tubular cells.

These findings highlight the protective nature of FBP for renal cells, improvement of tubular cell morphology and function, and reduction of reactive oxygen species production. Our results indicate that systemic delivery of FBP is a promising strategy for the treatment of AKI.

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Inhibition of Fas-mediated apoptotic signal by Fas-binding peptide ameliorates Parkinson's disease

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Parkinson's disease (PD) is a progressive disorder due to the death of dopamine secreting cells, which affects the nervous system and accompanying body parts. Currently, treatments are limited to managing symptoms, and no cure is available. Numerous studies suggest that the inhibition of Fas-mediated neuronal cell death, which consequently blocks the Fas-signal-protected neuron cells from degeneration and reduces oxidative stress, is a potential therapeutic strategy for PD.
Previously, we had administered Fas-blocking peptide (FBP) intranasally (IN) to the brain of an ischemic model and found FBP inhibited neuronal cell death. In this study, we examined whether IN administration of 2mg/kg FBP would inhibit neuronal cell death, oxidative stress, and inflammation in the mid-brain. To test the systemic delivery of FBP to the brain, a 30-amino-acid leptin peptide conjugated with PEGylated FBP (Leptin-PEG-FBP) was administered intravenously. The 2mg/kg Leptin-PEG-FBP was successfully transported across the blood-brain-barrier and ameliorated disease-associated symptoms in the PD mouse model. Inhibiting Fas-signaling reduced neuronal cell death in the mid-brain, resulted in the expression of tyrosine hydroxylase, which is involved in dopamine synthesis, and decreased apomorphine-induced rotations by 66%.
Our results indicate that the systemic delivery of Leptin-PEG-FBP is a promising strategy for PD.

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Furfural Valorization by Amine Transaminase-Catalyzed Transamination

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In recent years, great efforts have been made to develop more sustainable pathways of organic amine synthesis via biocatalyst implementation. Amine transaminases (ATAs) play the main role in this endeavour as enzymes capable of transferring an amine group from an amine donor to an acceptor with a distal carboxylic acid group. Biomass-derived furfural is an abundant raw material, so its valorization is of great interest. Its transformation to furfurylamine has attracted much attention due to its numerous potential industrial applications, e.g., as an intermediate in the production of pharmaceutical compounds such as antiseptics, antihypertensives and diuretics.
In this work, an attempt was made to produce furfurylamine from furfural using various amine transaminases and different amine donors, namely isopropylamine, D-alanine, and (S)-(−)-α-methylbenzylamine in a batch reactor. The reaction was followed using HPLC, NMR and *in-situ* FTIR analysis. Only when (S)-(−)-α-methylbenzylamine was used as an amine donor, furfurylamine was produced, while isopropylamine and D-alanine yielded different Schiff bases and no desired product. Moreover, a spontaneous reaction of furfural with the furfurylamine was observed in the case when D-alanine was used as an amine donor.

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Self-Assembled Structures Based on His6-Tagged Amin Transaminase and Nanoparticles

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Nanomaterials exhibit significant promise when used as supports for enzymes immobilization. In this work, immobilization of His_6-tagged amin transaminase on metal-functionalized silicate nanoparticles was studied. The effects of nanoparticle size and linker length was analyzed. Besides, different metal ions, namely cobalt, gadolinium and iron were used for nanomaterials functionalization. The evaluation of immobilization efficiency and retained enzyme activity was performed, as well as the scanning electron microscopy (SEM) of the nanomaterial before and after enzyme immobilization.
The results show that smaller particles and longer linkers result in higher immobilization yields. The highest observed immobilization yield was 77%, while immobilization efficiency of 44.8% was achieved using 250 nm particles with the longest of the tested linkers. Among metal ions, cobalt resulted in the highest immobilization yield and efficiency. SEM analysis confirmed that self-assembled structures of enzymes and nanoparticles were formed.

Keywords: amine transaminase, nanoparticles, self-assembly, immobilization, biotransformation

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Theoretical study on Alanine-$[K(H_2O)_2]$+ complex: structures and intermolecular interactions

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K^+ is one of the most abundant ions in living systems and plays a crucial role in several processes such as regulation of cellular electrolyte metabolism, electric signaling in cells, transport of essential nutrients, and regulation of enzyme activity. Potassium channels are widely distributed in cell membranes, where they regulate the entry of potassium ions in the cell and play a selective role, for example by excluding sodium ions. The different hydration state of Na^+ and K^+ in the channel inner pore is a basic factor determining ionic selectivity. Here, we present a detailed study based on Density Functional Theory, on the rotational energy profiles of the water molecules in the most stable complexes of alanine – $K(H_2O)_2^+$. Despite $K(H_2O)_2^+$, where height rotational barriers are almost negligeable, the interaction with alanine permits to increase the height barriers, isolating the equilibrium forms. Results are discussed in view of molecular dynamics applications. Future works will concern studies on larger systems such as amino acid chains of interest for ion channels.

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Preliminary morphological and electrophysiological characterization of human primary endometrial cells

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The endometrium is characterized by an epithelium of cylindrical and stromal cells. The alteration of processes related to hormonal signaling or intracellular signals are critical for the physiological response during the ovarian cycle but also in pathological processes: adenomyosis/endometriosis. We have taken into consideration the process of migration/invasion which is strictly dependent on the activity of plasma membrane ion channels. We describe primary endometrial cell culture preparations, taken from 7 human uterine biopsies. Morphological analysis describes a polymorphic shape. On a few cells, we performed the electrophysiological characterization, patch-clamp technique, highlighting the presence of an outward current, characterized by noise behavior at high voltage pulse (major of 100 mV with 100 nM intracellular calcium free), indicating the involvement of big conductance calcium activated potassium (BKCa) channel. To develop a model closer to the in vivo situation we set up a CAM engraft model in chick embryo to evaluate the angiogenesis. These preliminary studies are mandatory to the extensive work to understand the role of BKCa channels in the migratory and invasion process in physiological (embryo implantation) and pathological (adenomyosis/endometriosis) conditions.

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ClEver Balloon: An Everolimus Coated Balloon For Treatment Of Vascular Stenosis – An In Vitro Evaluation

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Introduction: Drug coated balloons (DCB) are used for treatment of vascular stenosis and prevention of neointimal hyperplasia. Everolimus is a drug used on drug eluting stents. This comparative study evaluated the in vitro effects of everolimus, paclitaxel and retinoic acid (RA) on proliferation, viability and migration of human coronary artery smooth muscle cells (HCASMCs) and on promyeloblasts of the HL-60 cell-line.
Materials & Methods: MTT and Trypan blue exclusion assays were used to evaluate cell proliferation and viability after 24, 48 and 72 hours of treatment with 0.1, 1, 10 and 100 μM of everolimus, RA and paclitaxel. Scratch wound assay was selected to assess the effect of these drugs on cell migration of HCASMCs in an 8-hour frame.
Results: Everolimus significantly reduced the proliferation rates of HCASMCs and HL-60 cells after 72 hours in the 1 and 10 μM doses without affecting their viability compared with the strong effect of paclitaxel. The 100 μM dose of all drugs showed the most prominent effect on proliferation and viability. Lastly, everolimus significantly inhibited cell migration in all doses.
Conclusion: Everolimus demonstrates strong inhibitory effects on cell proliferation and migration highlighting its potential as drug on DCBs.

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Immobilized β-N-Acetylhexosaminidases in Applied Biocatalysis

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The presented research aimed to combine chemical synthesis with enzymatic transformation to obtain pure α-anomers of aromatic substituted galactosides as important chromogenic/fluorogenic substrates for rapid enzyme-based diagnosis. The process begins with the chemical synthesis of an anomeric mixture of α/β-substituted N-acetylgalactosamine (GalNAc) derivatives. Then, the β-anomer is selective hydrolysed to aglycon

and glycosidic residue GalNAc using fungal β-N-acetylhexosaminidases (EC 3.2.1.52, GH 20, Hex), exo-glycosidases with a broad substrate specificity. The compounds resulting from the enzymatic hydrolysis of the β-anomer have different physicochemical properties compared to the remaining α-anomer of the glycoside; thus, they are easily separated from the resulting mixture and reused for a glycoside synthesis.
In this research, free and immobilized forms of Hex from the genus *Penicillium* were successfully applied in selective hydrolysis of β-anomers of GalNAc derivatives. The enzyme was immobilized in lens-shaped polyvinyl alcohol hydrogel particles and used for repeated runs. Notable traits for the immobilized hexosaminidases are enhanced stability, reusability and convenient separation by filtration from the reaction mixture.
This work was funded by the Agency for supporting research and development, the agreement Nr. APVV-20–0208.

Keywords: β-N-acetylhexosaminidase, anomer separation, immobilization

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Probiotics Influence the Crosstalk Between Intestınal Epithelial Cells and the Immune System

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Intestinal inflammation depends on the imbalance of homeostasis between intestinal microbiota and immune system. Evidence shows that mast cells (MCs) play an important role in the function of the intestinal epithelial barrier. Probiotics have positive immune balance remedial effects and help establish conditions of immune tolerance. The study is aimed at investigating the role of a specific probiotic combination (Serobioma®,Bromatech S.r.l.,Italy) on the communication between immune and epithelial elements. The in vitro transwell model was employed and Caco-2 and HT29 human colon adenocarcinoma cells in single cultures and in coculture, with or without MCs, are used to reconstitute the intestinal epithelial barrier. The cells were challenged with the lipopolysaccharide proinflammatory stimulus and treated with the probiotic formulation. In order to evaluate the integrity of the epithelial barrier, transepithelial electrical resistance measurement was performed. The levels of cytokines released were evaluated using ELISA analysis. Results confirm that MCs are fundamental barrier elements for physiological intercellular communication and their presence can significantly influence barrier function and intestinal homeostasis. Synergistic or additive actions of bacterial strains are observed as result of the multi-strain probiotic composition, probably by trophic cooperation between strains.

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Production of Protein-rich, Easily Metabolizable Biomass from Phanerodontia chrysosporiumA-1 using Apple Juice Industry Waste

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The environmentally safe and cost-effective biotransformation of apple juice industry waste into protein-rich and easily digestible biomass was the aim of the presented study.
The test object for the waste fermentation – the strain of filamentous fungi *Phanerodontia chrysosporium* A-1 was selected from the collection kept at the S. Durmishidze Institute of Biochemistry and Biotechnology, Agricultural University of Georgia.
Apple pomace (the residue of apple juice industry) – the substrate for fermentation was supplied by the "Kula" company (Georgia). The optimal conditions for solid-phase cultivation of *Ph. chrysosporium* A-1 were established (cultivation 10 days, at 40°C, with pH5 of the nutrient medium). On the base of optimization of the nutrient medium composition of *Ph. chrysosporium* A-1 the optimal amount of bioconversion substrate and the best nitrogen source (NH_4NO_3), as well as its optimal concentration (36 mg per g of the substrate) has been selected. Based on the optimization of strain cultivation conditions and the nutrient medium composition, a partially delignified, easily digestible, protein-rich (20,9%) biomass was obtained. The content of hardly degradable biopolymer lignin in the biomass decreased by 47.7%, cellulose - by 49.5% and hemicellulose - by 43.8%.

Keywords: solid state fermentation, mycoprotein, protein-rich biomass, apple pomace

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Site-specific Immobilization of Phenylalanine Ammonia-lyase from Arabidopsis thaliana

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The development of synthetic processes based on biotransformations has advanced significantly in recent years, enabling technologies with increased efficacy, productivity and selectivity, reduced costs and environmental footprint. Besides high catalytic activity and selectivity, decisive factors for the successful application of enzymes are stability and recyclability, generally achieved through immobilization. Due to the recent advances in protein engineering and structural analysis, efficient site-specific enzyme immobilization techniques have emerged to overcome the limitations of non-specific covalent immobilization, providing increased biocatalytic activity. A simple, versatile and efficient approach for site-specific enzyme immobilization, employs the maleimide/thiol coupling of engineered enzymes with cysteine residues introduced at specific positions

on the enzyme surface, to maleimide-functionalized supports1. Phenylalanine ammonia-lyase from *Arabidopsis thaliana* (*At*-PAL), naturally catalyzing the non-oxidative deamination of L-phenylalanine to *trans*-cinnamic acid, recently proved to be superior to other well-studied PALs in the biotransformation of several substituted phenylalanine and *trans*-cinnamic acid analogues.
Motivated by the previous successful site-specific immobilization of PAL from *Petroselinum crispum*, we focused on developing the site-specific immobilization of *At*PAL.

Keywords: site-specific enzyme immobilization, phenylalanine ammonia-lyase, phenylalanines.

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Human Carbonic Anhydrase a Powerful Enzyme in Chemical Biotechnologies

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Carbonic anhydrases (CAs) are mostly zinc-containing metalloenzymes, catalyzing the reversible hydration of carbon dioxide and dehydration of bicarbonate in a two-step ping-pong mechanism. The CAs have been extensively studied due to their broad physiological importance in all kingdoms of life and clinical relevance as drug targets.
The reactivity and selectivity of the enzymes can be modified by changing the donor/acceptor character of the ligands. Carbonic anhydrases is a class of naturally occurring metalloenzymes in which the native Zn^{2+} has been replaced by other transition metals such as Ni^{2+} or Co^{2+}. The aim of the research is the asymmetric reduction of ketones with silanes catalyzed by hCAII to form chiral alcohols enantioselectively. Different substrates were tested to monitor enzyme activity, selectivity and substrate tolerance.
It was demonstrated that hCAII is a versatile biocatalyst for the abiotic reduction of variously substituted phenyl-alkyl-ketones. While the nature of the substrates had a slight impact upon the activity of the enzyme, the stereoselectivity of the reaction was strongly influenced by the size and the polarity of substituents.

Keywords: hCAII, abiotic reduction

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Enzymes in Biocatalysis: Unlocking their Potential for Sustainable Chemical Synthesis

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This study delves into the remarkable adaptability and tolerance of two enzymes, which have showcased their prowess as whole-cell biocatalysts. In this work, we explore the development of a novel bi-enzymatic cascade system comprising *Pseudomonas psychrotolerans* ω-transaminase (*PpS*-TA) and human carbonic anhydrase II (hCAII) to implement the efficient conversion of various (±)-1-phenylethan-1-amines into their corresponding enantiopure (*R*)-amines and (*S*)-alcohols. The research demonstrates the enzymes' enhanced flexibility across a spectrum of reaction conditions. The reaction sequence involves the complete transformation of the reactive stereoisomer of (±)-1-phenylethan-1-amine (up to 40 mM) into acetophenone, catalyzed by soluble *PpS*-TA in the initial stage, followed by the stereoselective reduction of the formed ketone to (S)-1-phenylethan-1-ol in the presence of hCAII. Comparatively, the whole-cell cascade approach exhibits the capability to scale up the conversion of (±)-1-phenylethan-1-amine (1.2 g - 100 mL reaction mixture) to enantiopure (*R*)-amine and (*S*)-1-phenylethan-1-ol, yielding over 93% of the desired products.

Keywords: ω-transaminase, human carbonic anhydrase, bi-enzymatic cascade

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Immobilization of Bacillus subtilis Spores in a Miniaturized Reactor by a Magnetic Field

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Efficient biosensors for monitoring bioactive molecules such as antioxidants and phenols are in high demand. Conventional methods such as HPLC detection are time consuming and often insufficient to assess the cumulative capacity of antioxidants in biological samples due to the synergistic effect of the components. Bacteria-based sensing systems offer selectivity, sensitivity, and ease of use, but their limited long-term viability under extreme conditions limits their broad application.
To overcome these challenges, *Bacillus subtilis* spores, which are extremely resistant to harsh environmental conditions, can be used. In the endospore coat the CotA with the laccase activity is present. The sensor detects the conversion of colourless ABTS to the chromophore radical $ABTS^{*+}$ in the presence of oxygen. The use of endospores instead of purified enzymes reduces costs and processing time.
In this study, we upgraded the batch biosensor system to a flow-through system. The magnetic field assisted two-plate microreactor system was developed and its continuous flow performance was tested. Immobilisation efficiency was investigated by varying the ratio of microparticles to spores and buffer pH. Minimal spore leakage (less than 2%) occurred within 5 h, confirming the suitability of this configuration for the development of *B. subtilis* endospore biosensors.

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Comparative Analysis of metabolites/neurotransmitters in Ovarian Follicular Fluid: A Study on Polycystic Ovary Syndrome (PCOS) Patients Undergoing IVF Treatment

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This study aimed to investigate the presence and levels of metabolites/neurotransmitters such as L-glutamic acid, L-aspartic acid and Noradrenaline (NA) in the ovarian follicular fluid of polycystic ovary Syndrome (PCOS) patients compared to a control group. The study included 20 patients undergoing in vitro fertilization (IVF) treatment, with 10 individuals diagnosed with PCOS and 10 in the control group. Liquid chromatography mass spectrometry (TOF) was employed to analyse the follicular fluid, revealing the identification of 333 metabolites/neurotrasmitters. A preliminary the statistical analysis was conducted using MetaboAnalyst, focusing on L-glutamic acid, L-aspartic acid. No significant differences were observed between the two groups (p-values of 0.3 and 0.4). Additionally, neurotransmitters such as Noradrenaline is not detected in the FF, it might be a consequence of degradation pathway. Same results was obtained with TARGET approach HPLC-MS. Based on these findings, it can be inferred that there is no discernible difference in some amino acid neurotransmitters L-glutamic acid and L-aspartic acid in the follicular fluid of PCOS patients compared to non-PCOS patients. Further research and investigations are warranted to comprehensively understand the role of neurotransmitters in PCOS and their potential implications in the context of IVF treatments.

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Increased level of Vasoactive Intestinal Peptide (VIP) in follicular fluid of patients with polycystic ovary syndrome during IVF

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Polycystic ovary syndrome (PCOS) is a common multifactorial and polygenic disorder of the endocrine system, affecting between 5 and 10% of women of reproductive age with a still unknown etiology. Follicular fluid (FF) represents a *milieu* necessary to normal development of follicles rich of metabolites, hormones and neurotransmitters but in some instance of PCOS the composition can be different. Vasoactive intestinal peptide (VIP) is an endogenous autonomic and sensorial neuropeptide involved in follicular atresia, granulosa cell physiology and steroidogenesis and in some aspects of gonadotropin independent phase. We found an increase about 40% concentration of VIP in FF of PCOS patients (n=9) undergoing IVF procedures respect to non PCOS (n=10). We also found in FF of PCOS patient recovery samples with higher VIP concentration (>150 picograms/ml) indicates the presence of a subpopulation in PCOS with higher VIP concentration values with respect to control group. A direct correlation was found between the VIP FF concentration and plasma Anti-Mullerian Hormone (AMH) concentration (p=0.05 and a correlation index of 0.45). The relationship between VIP and AMH levels in PCOS pathophysiology is also discussed.

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Classification of Alzheimer's Disease with 3D CNN-LSTM on MRI Based on Time Difference

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In this study, the goal was to design and implement a deep learning model for the analysis of 3D MRI brain scan sequences to differentiate between 22 Alzheimer's Disease (AD) and 18 healthy controls (CN).

The MRI data, which is stored in NIfTI format, was sourced from an organized dataset, cataloging metadata such as image identifiers, patient names, class labels, and sex information. The data extraction procedure was meticulously structured: MRI scans were first segregated by unique subjects, then resized to a consistent target size, and finally organized into sequences for each patient. To effectively capture the longitudinal variations in the MRI scans across 3-4 different time points from each patients, a Long Short-Term Memory (LSTM) network was in-

tegrated into a pre-trained 3D ResNet architecture.
Emphasizing the significance of time-differentiated patterns, this combined network was engineered to handle the sequences of 3D images, thereby enhancing its capability to discern between AD and CN based on the temporal dynamics inherent in the MRI sequences. We have achieved accuracy of %94 on classification of AD and CN with %10 higher than classification with 3D ResNet.

Keywords: MRI, Alzheimer's disease, 3D, CNN, LSTM

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Probiotic and Antioxidant Properties of *Lactiplantibacillus plantarum* DB2 Isolated from Strawberry (Fragaria x ananassa Duch. cv. 'Albion')

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Probiotics, living organisms that provide health benefits when consumed in sufficient amounts, play an important role in improving human health. Probiotic microorganisms are increasingly recognized for their beneficial effects on various aspects of health, driving the popularity of probiotic-rich foods. Due to the challenging conditions in fruit matrices that make it difficult for probiotics to survive over time, it is critical to identify probiotic strains that are well-suited for these conditions. Such strains are well-positioned candidates for fruit-based probiotic products.
In this study, the probiotic traits of the strawberry isolate *Lactiplantibacillus plantarum* DB2 were investigated. The investigation encompassed its endurance under gastrointestinal conditions, antimicrobial capabilities, biofilm formation, and cell surface hydrophobicity. Additionally, the safety criteria of hemolytic activity and antibiotic susceptibility were tested. DB2 showed high survival rate in simulated gastric conditions, strong biofilm formation ability and high surface hydrophobicity. The strain also showed high inhibitory activity against the pathogens tested with exceptionally high inhibition of *Listeria monocytogenes* (74%). DB2 met all safety parameters tested.
This study presents for the first time the probiotic characterization of a *Lactiplantibacillus plantarum* strain isolated from strawberries and could be the first step towards the development of new functional probiotic products.

Keywords: probiotic, strawberry fruit, *Lactiplantibacillus*, characterization

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