POSTER PRESENTATION ABSTRACTS

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A physico-chemical study on trans-Resveratrol - Cu(I) complex: a theoretical approach

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Resveratrol is popular for being a component of red wine and for its correlation with anti-tumor and cardioprotective properties [1]. It is widely employed in nutraceutics production. It presents stilbenoid structure; in nature there are two isomers, cis and trans, this latter is the most stable. In this work, we present a preliminary theoretical investigation on the most stable complex that trans-resveratrol forms with Cu (I), an essential trace element in living organisms, that takes part to redox processes, where resveratrol plays an antioxidative role. Here, the structure of trans-resveratrol - Cu(I) is optimized in vacuum and in solvents like water and ethanol, by Density Functional Theory methods. Dissociation and solvation energies of the complex are calculated [2]. Infrared spectra are calculated and compared with experimental data, available in literature.

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Keywords: Resveratrol, infrared spectra, density functional theory

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Habitat-dependent variations of Chaetopterus variopedatus polychaete luciferases

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The marine polychaete Chaetopterus variopedatus is a cosmopolitan species complex, consisting of distinct populations. The worm's bioluminescence is confined to brightly glowing parapodia and clouds of mucus released upon stimulation. It has been previously proposed that the Fe^{2+}-dependent C. variopedatus luciferase may be used for the detection of ferroptosis – a pathway of programmed cell death, resulting from accumulation of ferrous ions. The current investigation was aimed at extraction and characterization of the Chaetopterus luciferases, along with a comparative study of luciferases obtained from different populations of these polychaetes. The enzyme extraction from the frozen samples of Brazilian C. variopedatus yielded two active luciferases, termed L1 and L2. We assumed that one of the luciferases is responsible for luminescence of the mucus while the other for luminescence of parapodia, and applied the developed purification method to the distinct samples of mucus and parapodia of the living Far Eastern C. variopedatus. However, mucus of the latter turned out to be non-glowing. Thus we have shown that luciferase L2 isoform defines Chaetopterus parapodia luminescence, while light production of the Brazilian C. variopedatus mucus is attributed to the functioning of luciferase L1. This study was supported by Russian Science Foundation, grant no. 18-74-10102P

Keywords: Bioluminescence, polychaetes, Chaetopterus variopedatus, luciferases

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Enzymatic Hydrolysis at High Solids Loading of Deacetylated Rice Straw

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Rice straw is considered as a promising feedstock for 2G ethanol production in biorefineries. However, one of the main challenges is to produce high concentrations of fermentable sugars. In this work, we evaluated the saccharification efficiency at high solids (20% w/v) of rice straw deacetylated with dilute alkali. Assays were carried out for 49 h at 50°C and 100 rpm varying the loads of Cellic Ctec2 from 10 to 45 mg protein/gcellulose). It was showed that, in Erlenmeyer flasks, increasing enzyme dosage, the glucan and xylan conversion was increased ~46%, being achieved maxima conversion of 72 and 52%, respectively, with 30 mg protein/g cellulose. In this condition, the

total fermentable sugars were of 98 g/L. As a strategy to improve hydrolysis at high solids, a novel reactor design (vertical ball mill reactor) was assessed. At 30 mg protein/g cellulose, the glucan and xylan conversion in the reactor were improved to 85 and 62%, respectively, producing 117 g/L fermentable sugars. This study showed that the reactor design plays a key role on the enzymatic hydrolysis step at high solids loading. Further studies will be addressed to developing an adequate enzyme formulation, especially with addition of xylanases aiming to improve hydrolysis yield

Keywords: Rice straw, enzymatic hydrolysis, high solids loading, fermentable sugars

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Chemical and rheological characterization of biobinders derived from different lignocellulosic biomass sources

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Researchers in Biotechnology and Civil Engineering fields have been increasingly concerned with developing eco-friendly construction materials to reduce environmental and economic impacts caused by the construction industry. One of the potential alternatives is the production of biobinders using in pavement applications. Biobinders are materials produced from renewable resources such as by-products of lignocellulosic biomass. In this context, this study aimed to evaluate the use of sugarcane bagasse and rice husk to obtain biobinders as total or partial substitutes of petroleum-based asphalt binders. Chemical and rheological characterization of biobinders were realized by elemental analysis, FTIR, and Frequency/Scanning tests. Among the biomaterials studied, biobinder derived from sugarcane bagasse showed higher stiffness in comparison with biobinder derived from rice husk. This result is directly related to their chemical composition, since the biobinder derived from sugarcane bagasse showed presence of fatty acids esterified, which was not observed in chemical composition of biobinder derived from rice husk. Furthermore, frequency scanning test showed that rheological behavior of biobinder derived from rice husk was closer to the petroleum-based asphalt binder (AC 30-45) at temperatures of 60 °C and 70 °C, this result indicated that this biobinder could have similar characteristics to petroleum asphalt binders at high temperatures.

Keywords: Biobinders, lignocellulosic biomass, pyrolysis, asphalt, sustainable development

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Exosomal ceramide mediates the action of vitamin D

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It has been demonstrated that vitamin D3 affects sphingolipid metabolism and vice-versa. It stimulates the sphingomyelin breakdown to produce ceramide. Supplementation with vitamin D3 in overweight/obese African American patients resulted in a high level of ceramide serum. Moreover, it has widely described that sphingolipids are involved in the formation of exosomes which consists in invagination of the plasma membrane, formation of intracellular multivesicular endosomes, fusion of these with the plasma membrane and their release in extracellular environment. We have previously demonstrated that the vitamin D3 is able to induce embryonic hippocampal cell differentiation by increasing the exosome release. Moreover, we showed that exosomes released under Vitamin D treatment were richer in sphingolipid and particularly in ceramide content. here we have treated embryonic hippocampal control cells with the same ceramide concentration present in exosomes released after vitamin D treatment. The results show the same degree of differentiation stimulus than vitamin D, suggesting a specific role of exosomal ceramide in this process.

Keywords: Embryonic hippocampal cell, vitamin D, ceramide, exosomes

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Microbial Poly-β-hydroxyalkanoate Accumulation from Wastewater Sludge for Plastic Production

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Treated wastewater sludge may contain harmful compounds that prevent its use as fertilizer. However, wastewater sludge carbon could be used to produce biodegradable plastic material called polydroxyalkanoates (PHAs), which could replace the traditional plastics made from fossil raw materials to reduce carbon dioxide emissions. This takes place in a two-step process. First, the sludge is hydrolysed into volatile fatty acids (VFAs), and then in the second step, bacteria enriched from the activated sludge convert the VFAs into PHAs in an aerat-

ed bioreactor. For the hydrolysis, pH values of 9, 7, 5, and no pH adjustment were tested in high volatile solid (VS) and high fixed solid (FS) bioreactors. Based on the VFAs yield, pH 7 was chosen for the sludge hydrolysis experiments. Three types of sludge (sludge after nitrogen removal, sludge for biogas production, and dewatered sludge) were hydrolysed at pH 7. The sludge intended for biogas production produced the maximum amount of VFAs (8861 ± 323 mg/L). In PHA accumulation, the feast-and-famine process was not suitable for converting VFAs to PHAs in the sludge with chemical phosphorus precipitation, but fermenter aeration had to be continued to cultivate PHA-accumulating microorganisms. The final PHA concentration was 80% of volatile solids.

Keywords: Polydroxyalkanoates, Volatile fatty acids, Wastewater sludge, pH

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The preliminary promising results on metabolic enzyme inhibitory activities of aryl substituted quinolines

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In drug discovery, quinoline derivatives have been highly attractive ring systems in organic chemistry owing to their well-documented pharmaceutical properties and wide range of medicinal benefits. The aims of this study are to determine the activities of them and the recently prepared substituted phenyl quinolines against Acetylcholinesterase (AChE) and Charbonic anyhydrase (CA) enzymes. The recent prepared phenyl substituted tetrahydroquinolines and quinolines were screened for human carbonic anhydrase I, II isoenzymes (hCAs I and II) and AChE inhibitory activities. In vitro inhibition of enzyme were evaluated against cytosolic hCA I, hCA II and AChE using the esterase assay and Ellman's method, respectively The results indicated that all the synthetic compounds exhibited potent inhibitory activities against all targets as compared to the standard inhibitors, revealed by IC50 values. Ki values of 5-, 6-, 5,7- and 6,8-disubstituted (trifluoromethoxy, thiomethyl and methoxy) phenyl quinolines and for hCA I, hCA II and AChE enzymes were obtained in the ranges 0.31-12.44, 0.92-12.45, and 8.56-27.05 μM, respectively. As a result, the preliminary experimental data indicated mono and diaryl substituted quinoline derivatives are potentially valuable drug candidates for the treatment of some diseases such as gastric and duodenal ulcers, glaucoma, epilepsy, osteoporosis, neurological disorders and Alzheimer's disease.

Keywords: Quinoline, Arylated Quinoline, Alzhemier Disease, Acetylcholineesterase, Carbonic Anyhydrase

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Screening of non-conventional yeast strains on mixtures of fish farm effluents with agro-industrial wastewaters

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Non-conventional yeasts gain increased attention thanks to their natural characteristics (e.g., thermo- and osmo-tolerance, salt resistance, etc.). However, research on their multiple applications is still scarce. In this context, the presented investigation aimed to identify yeast strains able to grow in oligotrophic environments of high salinity and/or pH values at different temperatures. Thus, 1065 strains were cultivated at 15, 25, and 37 °C on the liquid medium that remained after microalgae cultivation on fish farm effluents (FFEs). Interestingly, 10.0, 8.1, and 25.7% respectively grew satisfactory. The strains able to grow at 15 and 25 °C were also cultivated on FFEs enriched with pomegranate residues, which worked as a carbon source (containing 22.3 g/L of reducing sugars). From these, 21.9% grew at 15 °C and 25.8% at 25 °C, offering the potential for the simultaneous valorization of two wastewaters in northern and Mediterranean European countries. Those which grew at 37 °C, which are by definition opportunistic pathogens, were also cultivated on a mixture of FFEs and olive mill wastewaters, an agro-industrial residue rich in phenolic compounds (i.e., 5.3 g/L), and 33.3% was able to grow. In conclusion, non-conventional yeasts offer a variety of new applications that need further investigation.

Keywords: Non-conventional yeasts, fish farm effluents, pomegranate residues, olive mill wastewaters, opportunistic pathogens

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Effect of organic fertilizer from wheat bran and treated coffee grounds on plant growth

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World production of wheat bran reaches 100 million tons per year and spent coffee grounds about 6 million tons per year. This material often remains unused and is landfilled. Various strategies can be adopted to minimize landfilling of food waste, because food waste has interesting properties. The long-term application of food waste organic fertilizer was shown to improve soil quality. However, it was previously reported that the addition of 2,5 % of spent coffee grounds to the soil has a significant negative effect on plants. Therefore, our study is focused

on the detoxification of spent coffee grounds and its subsequent use as a fertilizer in combination with native wheat bran and thermally treated wheat bran. In the experiments, the content of phenolic substances and caffeine was reduced by oxidation and extraction procedure. Results obtained during the duration of the experiments have shown that despite the reduction of phenolic substances and caffeine in coffee spent grounds after detoxification procedure, the soil containing treated spent coffee grounds harmed plant growth even though the partial physicochemical parameters of the soil were improved by the addition of organic fertilizer.

Keywords: Coffee grounds, food waste, detoxification, growing media, biofertilizer

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Salt-Assisted Liquid-Liquid Extraction- An Easy, Efficient, and Economic Approach towards Downstream Processing of Fermentative Itaconic Acid

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Itaconic acid (IA) is a promising green substitute for various fossil-based platform chemicals applied in the polymer industry. The fungal fermentative process involving Aspergillus terreus is harvest grounds for scalable IA production. The currently employed downstream processing approaches are tedious and majorly contribute to its high production cost that demands newer ways for its reduction. Besides utilizing waste feedstocks, employing simpler techniques for downstream recovery of IA could be instrumental in achieving more cost-effectiveness. The current study includes the development of an optimized method for a single-step recovery of IA from the fermentation broth of Aspergillus terreus NRRL 1960. Partitioning of IA with various polar organic solvents coupled with the salting-out effect of different inorganic salts was studied for its recovery from the aqueous solution. The highest partitioning of IA was observed with isobutanol when Na2SO4 was dissolved in the aqueous solution of IA. Further, more intricate investigations on the effect of different process parameters viz. salt strength, IA concentration, pH, solvent volume, processing time, and multiple time extraction were attempted. The final optimized method resulted in 85.70% IA recovery and 94.2% purity of the product extracted from fermentation broth.

Keywords: Itaconic acid, Solvent extraction, Downstream processing, IA recovery

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The metagenomic communities of Tuber aestivum collected in Russia

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Truffle mushrooms are one of the least studied groups of fungi. Here, we characterized the prokaryotic and eukaryotic organisms living in the Russian truffle related to Tuber aestivum for the first time. The rate of true truffle in the consortium of eukaryotic organisms varied from 42 to 54%. The sequencing of hypervariable fragments of 16S rRNA and 18S rRNA genes led to identify the plant pathogens, involved in the symbiotic relationships with truffle mushrooms. Many of the mentioned species of prokaryotic and eukaryotic organisms have influences of the representatives of truffle mushrooms and help them to form mycorrhiza with trees. Besides the truffle mushrooms, the representatives of bacteria, fungi, and protists were found in the fruiting bodies. We suppose that both truffle mushrooms, and associated organisms are the perspective and a little studied sources for modern biotechnological studies. The study was supported by project of RSF 20-76-00001, 22-76-10036. Infrastructure for research was achieved by project of Ministry of Science and Higher Education of the Russian Federation (project with state registration 121111100025-5 at 11 November 2021).

Keywords: Truffle, Tuber aestivum, microbial pathogens

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Heterologous Expression, Purification, and Biochemical Characterization of Two Polyextremophilic Proteases from Marine Halotolerant Exiguobacterium sp

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Marine halotolerant bacteria are the enormous reservoir for enzymes working under extremes. Such bacterial genomes could be screened for industrially significant extremozymes. Our halotolerant isolate's (Exiguobacterium sp. TBG-PICH-001) genome was mined for proteases and two novel enzyme sequences viz. metalloprotease (ZMEI) and serine protease (SPEI) were screened for further heterologous expression in pET 22b plasmid vector and E. coli BL21DE3 strain. The ZMEI and SPEI showed soluble expression with the molecular size of 45kDa and 32kDa respectively. These were purified through Ni-NTA affinity chromatography. They respectively showed the temperature optima at 37oC and 35oC whereas the pH opti-

ma were 8.5 and 9.0. The proteases were highly stable in the alkaline pH range and at high temperatures (45-55oC). These were extremely tolerant towards various metal ions and surfactants. The residual protease activity was completely lost in the presence of EDTA for ZMEI while SPEI showed similar results in the presence of PMSF. Their halotolerant origin attributed them to high stability towards high salt and various organic solvents. Such polyextremophilic proteases bearing attractive characteristics of halo, thermo, and solvent tolerance, could be instrumental in bioprocess and detergent industries performing efficient biotransformation besides being a tremendous cleansing agent and peptide synthesis tool.

Keywords: Halotolerant, Extremozymes, Heterologous expression, Polyextremophilic proteases

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The strain of Penicillium sp. isolated from Baikal amphipods is a source for screening of novel natural products with antibiotic activity

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In this study, for the first time, a strain of fungi related to the genus Penicillium sp. was isolated from Eulimnogammarus cyaneus, an endemic Baikal phytophagous amphipod species. This strain was cultivated in different liquid nutrient media, and then crude extracts of cell-free liquid culture and cellular biomass were analyzed using the HPLC-MS method. Dereplication of secondary metabolite profiles of the isolated strain led to the identification of known natural products, while many detected metabolites were not listed in the most extensive database. Most of the identified metabolites were characterized by biological activity. Our extracts demonstrated antibiotic activity against Gram-positive and Gram-negative bacteria. Thus, although the golden age of antibiotics ended many years ago and microscopic fungi are well-studied producers of known antibiotics, the inhabitants of the ecosystem of Lake Baikal possess great potential for the search for new natural compounds and the development of new drugs. The study was supported by project of Grant of President of Russian Federation (Project MK-1245.2021.1.4), and project of Ministry of Science and Higher Education of the Russian Federation (project with state registration No 121111100025-5 at 11 November 2021).

Keywords: Penicillium sp., Baikal, natural products, antibiotic activity

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Antibiofilm and antibacterial properties of nanotubular arrays on the surface of titanium as a material for orthopedic implants

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Along with the population aging, the demand for orthopedic implants is growing. A substantial problem associated with arthroplasties is the occurrence of prosthetic joint infections, as this type of infection is characterized by persistence and progressivity and is difficult to get rid of with conventional antibiotic therapy and often requires revision surgery. Therefore, research efforts are devoted to surface modifications of implant materials in order to improve the prevention of bacterial colonization and biofilm formation (representing a crucial pathological mechanism in infection development) while maintaining successful osseointegration. Nanostructuration is an effective surface modification technique that can adversely affect bacterial adhesion without impairing osteoblast function. In our work titanium foils possessing TiO_2 nanotubular arrays of nanotubes with two different dimensions were prepared and their antibacterial and antibiofilm properties were tested by cultivation with Staphylococcus aureus and Pseudomonas aeruginosa. Results showed reduced biofilm formation as well as cell viability in comparison to unmodified titanium surface, which is based on structural and physical properties (based on nanotube dimensions) of this modified surface without any participation of titanium ions.

Keywords: orthopedics, implants, titanium, surface nanotubes, antibacterial, antibiofilm

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High-cell-density fed-batch cultivation of Micrococcus luteus for enhanced carotenoid production

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Carotenoids are natural pigments synthesized by a variety of microorganisms, plants and animals. They are known for their yellow, orange and red color. Carotenoids have been shown to have many beneficial characteristics related to their antioxidant properties, including anti-inflammatory effects, and are therefore candidates for use in a wide range of medical applications. Carotenoids consisting of a chain of 50 carbon

atoms (C_{50}) have strong antioxidant and photoprotective properties, but have not been widely studied. The Gram-positive bacterium Micrococcus luteus is known to produce several C_{50} carotenoids, including sarcinaxanthin. However, fed-batch strategies for cultivating M. luteus at high cell densities, which is a prerequisite for economically viable microbial production, have not been reported to date. In this study, both pulse-based and continuous feeding strategies were developed. With these strategies, a biomass productivity of more than 1.5 g L^{-1} h^{-1} along with a high sarcinaxanthin product concentration was achieved.

Keywords: Micrococcus luteus, High-cell-density fed-batch cultivation, sarcinaxanthin

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Effects of carbon and nitrogen concentration on the antimicrobial proteins produced by Lactiplantibacillus paraplantarum

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Antimicrobial proteins and peptides (APPs) like bacteriocins, peptidoglycan hydrolases (PGHs) and ribosomal proteins produced by lactic acid bacteria (LAB) are important due to their activity against pathogens and undesirable microorganisms. APPs production depends on a number of factors such as carbon and nitrogen concentrations in the culture media. In this study, different concentrations of carbon and nitrogen were used to determine their effect on the antimicrobial activity (expressed as mm diameter of zone of inhibition per mg of protein) produced by Lactiplantibacillus paraplantarum isolated from a fermented meat. The identity of the strain was confirmed by the amplification of the recA gene and the protein profile obtained by mass spectrometry (MALDI-TOF-Biotyper) additionally to the 16s RNA. This strain inhibited the growth of Listeria innocua, Salmonella spp, Escherichia coli and Weissella viridescens. It was found that the antimicrobial activity was proportional to the concentration of nitrogen, being higher when using a nitrogen concentration of 5.7 g/L in the media culture. Two distinct APPs were identified as 50S ribosomal protein L14 (13.1 kDa) and N-acetylmuramidase (66.6 kDa) by mass spectrometry analysis (LC/MS-MS). Therefore, these APPs could be further characterized for its application ass bio-conservatives in the food and pharmaceutical industries.

Keywords: Carbon and nitrogen concentrations in the culture media, Antimicrobial proteins and peptides, Lactiplantibacillus paraplantarum

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Dry Heat Treatment and Milling Impact on Nutritional and Functional Properties of Red Sorghum

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Sorghum grain processing through heat treatment can determine an improved nutritional properties. This study aimed to evaluate the effects of dry heat treatment at two temperature (121 °C and 140 °C) and grain fractionation, on the chemical and functional properties of red sorghum flour with three different particles sizes (small, medium and large), for processes optimization. Results showed that the treatment temperature negatively influenced oil absorption capacity, swelling power, emulsion activity, protein and fiber, while the opposite trend was obtained for water absorption capacity, fat, ash, moisture and carbohydrate content. Sorghum flour particle size positively affected the water absorption capacity, emulsion activity, protein, carbohydrate and fiber content, while oil absorption capacity, swelling power, fat, ash and moisture were negatively influenced. The optimization process highlighted that treatment temperature at 133 °C induce in medium particles size of red sorghum grains an increase in fat, ash, fiber and carbohydrates content. These results can be useful for researchers and the food industry in order to development of new products.

Keywords: dry heat treatments, fractionation, functional properties, nutritional properties, sorghum grains

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Whole genome sequencing and functional analysis of a novel biofilm-eradicating strain Nocardiopsis lucentensis EMB25

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The ever-growing antimicrobial resistance (AMR) has emerged as one of the leading public health threats of the 21st century. According to the WHO and numerous other groups and researchers, AMR is an urgent issue for which a global, coordinated action plan is needed. The formation of biofilm is considered a universal strategy to survive in unfavorable conditions used by the bacteria ubiquitously. The thick exopolysaccharide layer protects the bacteria from various environmental stress, including quenching of antibiotics resulting in restricted penetration. Moreover, the transfer of antibiotic resistance genes between bacterial species also encourages bacterial tolerance

towards antibiotics, exacerbating the current AMR situation. In the current study, we have isolated a bacteria that has never been explored for biofilm eradication also did the whole genome analysis using the Illumina sequencing and the GridION X5 Oxford Nanopore Technologies. Phylogenetic analysis revealed that Nocardiopsis sp. EMB25 is closely related to the Nocardiopsis dassonvillei_NOCA502F. The gene prediction from the assembled genome was carried out using PROKKA tool. The predicted proteins were used for similarity search against the uniprot bacterial database using DIAMOND BlastP program for the gene ontology and annotation. 5393 proteins were annotated out of 5775 predicted proteins.

Keywords: Antibiotic resistance, whole-genome sequencing, Biofilm, actinobacteria

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"Hot" producers of polyhydroxyalkanoates as the future of the biotechnological industry

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Polyhydroxyalkanoates (PHA) are very interesting and promising materials microbial polyester which are produced by many various bacteria. Additionally, there are completely biodegradable and biocompatible, providing a wide range of end uses. However, production is expensive. Fortunately, PHA are also produced by extremophiles, on which the next generation of biotechnological production is based. Extremophilic organisms live in extreme conditions and due to this fact, the biotechnological process is not so demanding on sterility and the cost of production is lower. We focused on PHA production by bacteria that like hot - they are called thermophiles. PHA production has been confirmed in several species, but the genus Aneurinibacillus appears to be the most interesting due to its unique properties and ability to produce interesting and very unusual copolymers. Or also the bacterium Schlegellela thermodepolymerans a good producer of homopolymer containing 3-hydroxybutyrate but can also efficiently use xylose as a carbon source or various waste lignocellulosic sources for its production.

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Keywords: polyhydroxyalkanoates, thermophiles, Schlegellela thermodepolymerans, Aneurinibacillus

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Antiviral Effect of Ivermectin on the Veterinary Viruses

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Ivermectin (IVM), a chemically modified derivative avermectin B1 is a broad-spectrum antiparasitic drug. It has recently been reported to have in vitro antiviral activity against various viruses. IVM inhibits the transport of viral proteins into the host nucleus through inhibition of the nuclear localization signal mediated by importin α/β. We found IVM to inhibit 99.94 %, 94.38 %, 99.99 %, 100 %, and 99.99 % in titers for BRSV, BPIV-3, BoHV-1, BCoV and BVDV replication for the groups treated with 5 μM IVM, respectively. We also examined the antiviral effect of IVM at different replication stages of PPR virus, Lumpy skin disease virus (LSDV) and Sheeppox virus (SPPV), the number of infectious virions for LSDV, SPPV, and PPR virus were decreased by 99.82%, 99.87%, and 92.50% at the viral replication stage, 68.38%, 25.01%, and 0.0% at the attachment stage, and 57.83%, 0.0%, and 57.83% at the penetration stage, respectively. Future studies could be beneficial for better understanding the molecular mechanism of action and opportunities for clinical treatment. On the other hand, these studies have demonstrated that IVM can inhibit the replication of veterinary viruses and may be a potential drug for viral infection.

Keywords: Ivermection, Virus inhibition, Veterinary viruses, Respiratory viruses

[PP-021] DOI: 10.2478/ebtj-2023-0004

Potency and inoquity of P2Vac® a combined formulation of attenuated Sheeppox and PPR virus vaccines

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Control of Sheeppox (SPP) and Peste des petits ruminants (PPR) viruses depends on vaccination programs by live attenuated vaccines. We studied a combined formulation of SPP and PPR vaccines. SPP and PPR vaccine strains were separately propagated in Vero cell line. The lyophilized vaccine formulation (P2Vac, 1ml) included >102,5 /per doses of each strain, sucrose (0,5mg) and lactalbumine hydrolysate (0,25mg). Safety of the vaccine was demonstrated in mice experiments. Safety testing in sheep and lambs was according to European Pharmacopie, CFR9 and OIE manuals. The animals were not previously vaccinated against sheeppox and PPR and free of antibodies to these infections. No side effects were recorded either in adult sheep (n=8) or in lambs (n:8). Over dose experiment was

also resulted in safe indicating the inoquity of the combined formulation for all ages of sheep. For PPR, immunogenecity was demonstrated by ELISA while it was investigated by serum neutralization assay for sheeppox. All the vaccinated animals were seroconverted to PPR while neutralizing antibodies to SPPV were detected only in one sheep. Vaccinated animals were completely protected against pathogen challenge at 30 dpv. The success of SPPV-PPRV vaccine in sheep experiments was demonsrated.

Keywords: Combined Vaccine, sheeppox, Peste des petits ruminants

[PP-022] DOI: 10.2478/ebtj-2023-0004

Physiological and Genomic Analysis of Bacillus pumilus UAMX Isolated from the Gastrointestinal Tract of Overweight Individuals

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The study aimed to evaluate the metabolism and resistance to the gastrointestinal tract conditions of Bacillus pumilus UAMX (BP-UAMX) isolated from overweight individuals using genomic tools. Specifically, we assessed its ability to metabolize various carbon sources, its resistance to low pH exposure, and its growth in the presence of bile salts. The genomic and bioinformatic analyses included the prediction of gene and protein metabolic functions, a pan-genome and phylogenomic analysis. BP-UAMX survived at pH 3, while bile salts (0.2–0.3% w/v) increased its growth rate. Moreover, it showed the ability to metabolize simple and complex carbon sources (glucose, starch, carboxymethyl-cellulose, inulin, and tributyrin), showing a differentiated electrophoretic profile. Genome was assembled into a single contig, with a high percentage of genes and proteins associated with the metabolism of amino acids, carbohydrates, and lipids. Antibiotic resistance genes were detected, but only one beta-Lactam resistance protein related to the inhibition of peptidoglycan biosynthesis was identified. The pan-genome of BP-UAMX is still open with phylogenetic similarities with other Bacillus of human origin. Therefore, BP-UAMX seems to be adapted to the intestinal environment, with physiological and genomic analyses demonstrating the ability to metabolize complex carbon sources.

Keywords: Bcillus, GUT, Pangenome, WGS

[PP-023] DOI: 10.2478/ebtj-2023-0004

Cosmetic application of Spirulina extracts with photo chemoprotective effect

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Sun exposure has a number of harmful impacts on human health, including sunburn, pigmentation, photo-aging, immune reaction, and skin cancer. We therefore focused our work on obtaining UV-absorbing extracts from the cyanobacterium Spirulina and integrating them into cosmetic emulsions. Several extracts from different Spirulina samples were prepared and characterised. The profiles of fatty acids, phenolic compounds, chlorophylls, and carotenoids were assessed. For the examination of specific activities, a number of factors were looked into, including antioxidant activity, SPF, and critical wavelength. For evaluation of cytotoxicity the MTT assay on HaCaT cell line was performed. Last but not least, model cosmetic emulsions were made and sensory testing was performed. Based on the results of the investigation described above, we can conclude that the created extracts had a high concentration of active chemicals, strong antioxidant activity, and UV protection properties. Since extracts had no effect on cell viability in the range relevant to cosmetics, they were used further in model cosmetic formulas. The emulsions that were produced were stable and had good sensory properties.

Acknowledgments

This work was also supported by the project at FCH BUT no. FCH-S-22-7961 of the Ministry of Education, Youth and Sports of the Czech Republic.

Keywords: Spirulina, photochemoprotection, extracts, active compounds

[PP-024] DOI: 10.2478/ebtj-2023-0004

Characterization and utilization of extracts from Spirulina and Chlorella vulgaris in food products

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Microalgal and cyanobacterial biomass is a rich source of many diverse substances that can be used as food and medicines by humans. Due to this, the primary goals of the study were to characterize extracts from Chlorella vulgaris and Spirulina. There were several extraction techniques applied. The amount of active substances, including antioxidants, phenolic compounds, carotenoids and chlorophylls, was measured. The cytotoxicity of the extracts on human cells was determined using the MTT test. The food application was enhanced via an encapsulating technique, which offers sufficient physical and chemical protection against the outside environment. The encapsulation minimizes unpleasant taste, smell, and color while also assisting with stability throughout processing and storage. The

prepared liposomal particles were evaluated for liposome size, efficiency of encapsulation, and short- and long-term stability. The findings indicated that liposomal components and produced extracts are potential for further uses. The acquired knowledge can be applied to the creation of dietary supplements and fortified foods. Acknowledgments: This work was supported by the project Nr. FCH-S-22-7961 of the Faculty of Chemisty, Brno University of Technology.

Keywords: Spirulina, Chlorella vulgaris, extracts, encapsulation, food application

[PP-025] DOI: 10.2478/ebtj-2023-0004

Thermophilic gram-positive bacteria of the genus Aneurinibacillus: Producers of unique PHA materials

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The pollution by petrochemical plastics is a still-increasing global issue that can be partly solved by using eco-friendly materials produced by microorganisms, so called polyhydroxyalkanoates (PHAs). Thanks to their properties PHAs can replace conventional plastics in a wide range of applications. The most common PHA is poly(3-hydroxybutyrate). However, due to its high crystallinity, it is relatively stiff and fragile. Therefore, the incorporation of different monomers into the PHA structure leads to better properties and PHAs can compete with synthetic plastics even more. The aim of this work is to study PHA copolymers production by six newly isolated thermophilic gram-positive strains of the genus Aneurinibacillus. Synthesis was performed by lactones (γ-valerolactone, γ-hexalactone, and δ-valerolactone) and glycerol as a structural precursor of different PHA monomers. Bacteria have demonstrated the ability to produce diverse PHA materials containing 4-hydroxyvalerate (production up to 69.3 mol. % of the total PHA content), 4-hydroxyhexanoate (up to 31.9 mol. %) and 5-hydroxyvalerate (up to 47.1 mol. %). Furthermore, it was observed that the produced copolymers show better mechanical properties in comparison with poly(3-hydroxybutyrate). We assumed that bacteria of the genus Aneurinibacillus can be considered a promising strain to produce interesting PHA copolymers and will be studied further.

Keywords: Aneurinibacillus, thermophilic bacteria, polyhydroxyalkanoates, lactones

[PP-026] DOI: 10.2478/ebtj-2023-0004

Calcium chloride – an economic way to deal with polysaccharide contamination when isolating DNA from plant-based foods

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Plant polysaccharides had been labelled "prime interferers" in DNA isolation. If a DNA isolate is contaminated with polysaccharides, it is difficult to purify, because polysaccharides often co-precipitate with DNA. In this work we compare amplifiability of DNA isolated from fruit and herbal teas, fruit bars, purees and a smoothie by unmodified DNA isolation kit, the kit with calcium chloride precipitation of polysaccharides added to the protocol, and the kit with pectinase digestion of polysaccharides added to the protocol. We focused on three parameters: Concentration, purity and amplifiability of DNA in a qPCR assay with primers specific for plant ITS2 region. Results indicate that while DNA isolates obtained by isolation protocol with calcium chloride have lower concentration and purity compared to isolates obtained by unmodified kit, and similar purity compared to isolates treated with pectinase, they show better amplifiability. In case of DNA isolates from fruit purees, samples obtained by the protocol with calcium chloride were the only ones which were successfully amplified. We therefore conclude that precipitation of polysaccharides with calcium chloride during DNA extraction has a beneficial effect on the quality of DNA isolates. Acknowledgments: This work was supported by the project Nr. FCH-S-22-7961 Faculty of Chemistry BUT.

Keywords: DNA isolation, calcium chloride, qPCR, fruit

[PP-027] DOI: 10.2478/ebtj-2023-0004

How to produce PHB co-polymers in cyanobacteria?

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Cyanobacteria are ecologically extremely important phototrophic gram-negative which belong to the prokaryotes. Cyanobacteria synthesize many interesting metabolites such as glycogen, lipids, carotenoids or polyhydroxyalkanoates (PHA). PHA are biodegradable biopolymers with similar properties as petrochemical plastics and they occur in bacteria in granules. Numerous prokaryotes including cyanobacteria use PHA as storage of carbon and energy in form of intracellular granules. The most common type of PHA is poly-3-hydroxybutyrate (PHB). PHA can be produced by heterotrophic bacteria as well, such as Cupriavidus necator H16 or Methylobacterium rhodesianum. PHB raises the fitness of bacteria because it helps the bacteria to survive stress conditions – osmotic stress, UV radiation, freezing or high temperatures. Both strains are capable of co-polymer (co-polymer of 3-hydroxybutyrate (3HB) and 4-hydroxybutyrate (4HB)) synthesis in presence of its structural precursor - γ-butyrolakton. The composition of co-polymer differs in Synechocystis sp. PCC 6803 and Syn-

echocystis salina CCALA 192 and the composition of co-polymers also vary depending on the precursor concentration. Funding: This study was partly funded by the project GA19-19-29651L of the Czech Science Foundation (GACR). Zuzana Sedrlova is Brno Ph.D. Talent Scholarship Holder – Funded by the Brno City Municipality.

Keywords: PHB, Synechocystis, co-polymer

[PP-028] DOI: 10.2478/ebtj-2023-0004

Liposomal particles functionalized by antimicrobial active compounds

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Lipid nanoparticles have been developed with the progress in nanotechnology and their unique properties have made them one of the most widely used nanoparticles. The presented work is focused on two types of nanostructured particles, liposomes and PHB-liposome particles. Materials were functionalized by active compounds, thymol and vitamin E. Liposomes were overall tested for stability, gradual release of incorporated substances in the model environment and biological activity. Liposomal size and colloid stability were defined by dynamic light scattering and zeta-potential. Particles they were subjected to the characterization of gradual release using the spectrophotometric method into model environments. Antimicrobial activity and synergic effect against gram-positive and gram-negative strains of microorganisms were also evaluated. To assess whether prepared nanostructured materials are suitable for further cosmetic applications, a cytotoxicity assay on the HaCaT cell line was carried out. Functional liposomal particles showed promising results for various applications as cosmetic preservation and pharmaceutical antimicrobial products.

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Keywords: lipid nanoparticles, liposomes, antimicrobial compounds, encapsulation

[PP-029] DOI: 10.2478/ebtj-2023-0004

Next Generation of Bio-inoculants, their Preparation and Viability for the Agricultural Applications

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Azotobacter vinelandii is a plant growth-promoting rhizobacterium (PGPR), capable of synthesizing two different biopolymers with great application potential. Polyhydroxyalkanoates (PHAs) are accumulated in a form of intracellular granules, whereas alginate is produced extracellularly. In agricultural demands, the use of PGPRs as bio-inoculants increasing plant and crop yields can be an ecological alternative to conventional fertilizers. To protect bacterial cells and sustain long-term effect of inoculant application, we developed the novel concept in which bacteria are encapsulated into hydrogel-based carriers by using self-produced alginate. In this work, the bacterial culture was crosslinked with a gelation agent (calcium chloride solution) to form a hydrogel carrier with encapsulated bacteria. The produced alginate ranges between 1,67 – 4.9 g/l depending on the strain, whereas the content of PHA varies from 30 to 50% of cdw in all the strains. The stability and strength of the bacterial gels were compared by rheological analysis. Moreover, according to our results the bacteria entrapped in a gel matrix are viable and cultivatable, which confirms the feasibility and viability of the novel concept.

Keywords: PGPRs, alginate, PHAs, bioinoculants, encapsulation

[PP-030] DOI: 10.2478/ebtj-2023-0004

Physiological and genomic analysis of Bacillus pumilus UAMX isolated from the gastrointestinal tract of overweight individuals

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The study aimed to evaluate the metabolism and resistance to the gastrointestinal tract conditions of Bacillus pumilus UAMX (BP-UAMX) isolated from overweight individuals using genomic tools. Specifically, we assessed its ability to metabolize various carbon sources, its resistance to low pH exposure, and its growth in the presence of bile salts. The genomic and bioinformatic analyses included the prediction of gene and protein metabolic functions, a pan-genome and phylogenomic analysis. BP-UAMX survived at pH 3, while bile salts (0.2–0.3% w/v) increased its growth rate. This strain was capable to metabolize simple and complex carbon sources such glucose, starch, carboxymethyl-cellulose, inulin, and tributyrin; but the growth in the presence of tributyrin induced a differentiated protein profile. Genome was assembled into a single contig, with a high percentage of genes associated with the metabolism of amino acids, carbohydrates, and lipids. Antibiotic resistance genes were detected, but only one associated to beta-Lactam resistance related to the inhibition

of peptidoglycan biosynthesis was identified. The pan-genome of BP-UAMX is still open with phylogenetic similarities with other Bacillus of human origin. Therefore, BP-UAMX seems to be adapted to the intestinal environment, with physiological and genomic analyses demonstrating the ability to metabolize complex carbon sources.

Keywords: Bacillus, GUT, Pangenome, WGS

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Probiotic viability and content of phytochemicals in fermented white cabbage juice

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Fermented white cabbage juice is a rich source of antioxidants and bacteria with potential health benefits. In this work cabbage juice underwent pasteurization treatment using 4 different combinations of temperature and time (70 °C/3 min, 80 °C/2 min, 90 °C/1 min, 90 °C/0.5 min) to eliminate potential pathogens and to extend its shelf-life. As negative effect, the viability of present probiotic bacteria decreased due to pasteurization treatment. Then, four selected probiotic strains were individually and also altogether inoculated into pasteurized cabbage juice. Biologically active compounds, such as antioxidants, organic acids, and probiotic viability were analysed during 2-week storage at 8 °C. It was observed that low pH due to presence of especially acetic acid helps to preserve antioxidant activity of cabbage juice. On the other hand, probiotic viability was lower and lag phase of inoculated probiotics ($1 \cdot 10^5$ CFU/mL) in cabbage juice was prolonged in comparison with inoculation in MRS Broth medium. It was observed that L. plantarum CCM 7039 was the most suitable probiotic strain to enrich cabbage juice after pasteurization treatment.

This work was supported by the project Nr. FCH-S-22-7961 of the Faculty of Chemistry, Brno University of Technology.

Keywords: probiotics, viability, shelf-life, acidity, antioxidants

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Lignans in wine

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Among compounds beneficial for human health, wine contains certain amounts of lignans, namely lyoniresinol, isolariciresinol, secoisolariciresinol, and to a lesser extent matairesinol, lariciresinol, and syringaresinol. Lignan concentrations can vary depending on the particular wine type from negligible concentration to more than 3 mg/L. Generally, the source of lignans in wine may be wood used in enology in the form of chips or wooden barrels or the grape bunch itself. Our results based on LC-MS analyses show that lyoniresinol originates from oak wood, isolariciresinol can be released into wine from all parts of the grape bunch, secoisolariciresinol comes from the grape seeds and possibly also from the berry skins, low amounts of matairesinol may be released into wine from the grape skins, and syringaresinol comes from the wooden parts of both the grape bunch and the barrel. Therefore, the lignan content in wine can increase with maturation in contact with grape berries, seeds, or stems or with wood. Furthermore, the content of syringaresinol can be affected by the type of wooden barrel used, the content of matairesinol is affected by the grapevine cultivar, and the amounts of isolariciresinol and secoisolariciresinol are affected by both the cultivar and the year of growing.

Keywords: lignan, wine, grape bunch, wooden barrel

[PP-033] DOI: 10.2478/ebtj-2023-0004

The pulsed ultrasound-assisted extraction of pectin from grape pomace

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Recently, there are several unconventional sources of pectin extracted from vegetables and fruits which have a different pectin content, physicochemical and structural features. The grape pomace is one of the most significant by-products obtained in the wine industry and represents about 25% of the grape weight which contains skin, seeds and other solid parts by pressing process. Pectin structure is a decisive factor on its plant source, extraction technique, physicochemical characteristics and applications. Various techniques, including conventional and non-conventional extraction methods have been employed in order to extract pectin from different plant materials. An overwhelming effort is doing based on the ethical dimension of sustainability and "green chemistry". Therefore, the influence of pulsed ultrasound-assisted extraction of pectin from Rară Neagră grape pomace was investigated. The pulsed ultrasound assisted extraction of pectin from Rară Neagră grape pomace was modelled utilizing the Box-Behnken design with three parameters as follows: extraction time (20, 40, and 60 min), amplitude (20, 60, and 100%), and pH (1, 2, and 3). The optimal conditions for pectin extraction were amplitude of 100%, pH 1.8 for 60 min (8.94% pectin yield and 78.64 g/100 g of galacturonic acid content) for Rară Neagră grape pomace pectin.

Keywords: grape pomace, extraction, pectin, yield, galacturonic acid

[PP-034] DOI: 10.2478/ebtj-2023-0004

Salt mine metagenomics – biodiversity characterization and novel natural products identification

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Saline environments are habitats of salt loving group of microorganisms named halophiles. In our research, we decided to analyze the biodiversity and biotechnological potential of the Bochnia Salt Mine microbiome. The mine operated from the 13th century to 1990 and has been inscribed on the UNESCO World Heritage List. DNAs from 24 samples of brine were isolated and prepared for sequencing according to the 16S Metagenomic Sequencing Library Preparation protocol from Illumina. For four samples additionally shotgun sequencing was performed. There were statistically significant differences between microbiome biodiversity, between the mine levels. Ultimately, 16 unique MAGs were obtained from the shotgun sequencing data. In the tested metagenomes were detected also 47 BGCs with AntiSMASH, 2303 BGCs with DeepBGC and 52 genes encoding potentially interesting AMPs. In summary, the microbiome of the Bochnia salt mine is an extremely diverse and complex microbial community. During our research, we detected over a dozen of new MAGs. Our research also shows the potential of microorganisms from this habitat as a source of new natural products.

The presented research was co-financed by the European Union from the European Social Fund under the "InterDOC-STARt" project (POWR.03.02.00-00-I033/16-00).

Keywords: metagenomics, halophiles, salt mine

[PP-035] DOI: 10.2478/ebtj-2023-0004

Cinnamomum zeylanicum essential oil modulates an LPS-induced inflammatory response in vitro

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In recent years, there has been significant interest in the identification of plant-derived essential oils (EOs) with health-promoting properties and potential as medicinal agents. Salvia officinalis and Cinnamomum zeylanicum EOs have been shown to act as antimicrobial compounds. In this study, the potential anti-inflammatory activity of these EOs was evaluated in in vitro human and mouse models based on monocytic THP-1 cells and RAW264.7 macrophages. Biosafe concentrations were estimated following cell viability studies and then used to assess the activity of the EOs in LPS-induced inflammation, determined by modulation of the expression of pro-inflammatory genes with RT-PCR and the secretion of cytokines with ELISA. Cinnamomum zeylanicum EO emerged as a more potent regulator of the inflammatory immune response, as evidenced by the significant down-regulation of IL-6 and IL-1b expression and the noticeably reduced levels of IL-6, IL-1b and TNFa detected in culture supernatants. These results suggest that Cinnamomum zeylanicum EO holds potential as an anti-inflammatory agent and further studies are underway to investigate its properties and the mechanisms involved in its activity.

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Keywords: essential oil, inflammation, LPS, cinnamon, THP-1 macrophages

[PP-036] DOI: 10.2478/ebtj-2023-0004

Function of plant-derived anti-HER2 VHH-FcK recombinant antibody for HER2-positive breast cancer

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Overexpression of human epidermal growth factor receptor type 2 (HER2) protein is one of the prognostic factors to diagnose breast cancer, and HER2 is proto-oncogene located on chromosome 17, and overexpression of this oncogene plays an essential role in the growth and progression of certain violent types of breast cancer. This study demonstrated the expression of the anti-HER2 camelid single domain antibody VHH in tobacco plant. And we confirmed the function of recombinant VHH for the treatment of breast cancer. The anti-HER2 VHH was fused to human IgG Fc domain along with KDEL endoplasmic reticulum (ER) (VHH-FcK) in a gene expression cassette. The plant gene expression cassette was transferred to tobacco plants using Agrobacterium-mediated transformation. In conclusion, this study verified the possibility of anti-HER2 VHH-FcK as a therapeutic agent and suggests that the anti-breast cancer anti-HER2 VHH-FcK can be expressed, properly assembled and purified from plant expression system, which can be as an alternative system for antibody production.

Acknowledgements
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Keywords: Plant, Antibody, VHH, HER2, Fc-fusion protein

[PP-037] DOI: 10.2478/ebtj-2023-0004

Chinese cabbage plant cell factory for biopharmaceutical production

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EpCAM is a tumor-related antigen and is a cell surface glycoprotein expressed in colon cancer. EpCAM has been considered a potential target for tumor vaccines. In this study, Transformation was applied to produce genetically modified Chinese cabbage to express EpCAM-IgM FcK. The recombinant protein productivity was maximized by developing a safe and efficient plant cell factory system using a modified plant cell line expressing EpCAM-IgM FcK protein. In addition, plant seedlings expressing both EpCAM-IgM FcK and J-chain K were obtained by crossing. PCR confirmed that the EpCAM-IgM FcK gene present in the seedlings. Western Blot screened seedlings with high expression. Chinese cabbage protoplasts were obtained by successfully optimizing the protoplast separation protocol to perform single cell transcription analysis. Single cell transcription analysis was performed to reveal the cell types that highly express EpCAM-IgM FcK protein. Cell lines with high expression will be applied to plant cell factory systems to produce EpCAM-IgM FcK.

Acknowledgements
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Keywords: Chinese cabbage, plant cell, KDEL, EpCAM, Cancer

[PP-038] DOI: 10.2478/ebtj-2023-0004

Transient expression of Angiotensin converting enzyme 2 (ACE2) fused to the fragment crystallizable (Fc) of human immunoglobulin G1 tagged with KDEL ER retention signal in Nicotiana benthamiana

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Acute respiratory syndrome coronavirus SARS-CoV-2 causing COVID-19 has been rapidly spreading worldwide since it occurred in December 2019. SARS-CoV-2 infection occurs as the receptor-binding domain (RBD) of the coronavirus envelope spike protein binds to the angiotensin-converting enzyme 2 (ACE2) receptor. ACE2 is a cell membrane protein activated when it is cleaved and released from the cell surface. In this study, the recombinant ACE2 protein was produced in a transient Nicotiana benthamiana plant expression system. The ACE2 was cloned to a fragment crystallizable (Fc) tagged with the KDEL sequence, endoplasmic reticulum (ER) retention signal (ACE2-FcK) in pEAQ-HT transient plant expression vector. To design the recombinant protein structure of ACE2-FcK, the transmembrane region of ACE2 was removed using a transmembrane region prediction program. The proteins (100kDa) were successfully expressed and purified from leaves using protein A affinity chromatography. So This results suggested that the plant-derived ACE2-FcK has bioactivities as a therapeutic agent for coronavirus.

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Keywords: COVID-19, Plant, KDEL, ACE2-FcK, Transient expression

[PP-039] DOI: 10.2478/ebtj-2023-0004

Advanced microscopic characterization of Rhodospirillum rubrum with various PHA contents

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Polyhydroxyalkanoates (PHA) are microbial polyesters of hydroxyacids that are produced and accumulated in the form of intracellular granules by various microorganisms. It is generally known, PHA do not serve only as energy and carbon source for microorganisms, but it has additional advantages for the vitality of cells during stress conditions (low temperature, pH, etc). In our study, we were focused on Rhodospirillum rubrum

(DSM 467), Gram-negative bacteria revealing substantial metabolic versatility and adaptability. Because of the role of PHA in this bacterium with remarkable versatility in metabolic strategies in the context of the biological importance of PHA under various metabolic conditions, we focused on the fundamental characterization of PHA granules in cells of R. rubrum and its involvement in biophysical and biochemical properties of these cells by several microscopic techniques. We used advanced microscopy techniques to investigate of the morphology of cells with various PHA contents such as Fluorescence Life Time Microscopy (FLIM), Transmission Electron microscopy (TEM) and freeze-fracture cryo-Scanning Electron Microscopy (cryo-SEM). For complex information about granules of PHA in cells, we also used Atomic Force Microscopy (AFM).

This work was supported by a joint project from the Czech Science Foundation [GA21-15958L] and the Swiss National Science Foundation [205321L_197275/1].

Keywords: Rhodospirillum rubrum, polyhydroxyalkanoates, electron microscopy, fluorescent microscopy, atomic force microscopy

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Evaluation of various spectroscopic approaches to the in-situ characterization of photosynthetic pigments in Rhodospirillum rubrum

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Wherever the energy metabolism of microbes under photo heterotroph and photoautotroph conditions is concerned, qualitative and quantitative analysis of the involved photosynthetic pigments represents a crucial and irreplaceable experimental task. Performing this analysis without the need of pigment extraction from the biomass is still an open and attractive analytical challenge. Rhodosporillum rubrum is a bacterium with extraordinary metabolic flexibility which is studied for various biological mechanisms and it is also employed in numerous biotechnological processes. In this contribution, we will compare and evaluate various methodological approaches for the in-situ analysis of photosynthetic pigments in R. rubrum cultures. The study involves techniques of molecular spectroscopy (ATR-FTIR, RAMAN microspectroscopy, FT-RAMAN), as well as routine (turbidimetry) and advanced (diffuse transmittance) spectrophotometry. Specific contribution of the individual methods to the complex information on the pigment composition and content in the cells are discussed.

Acknowledgement:
This work was supported by a joint project from the Czech Science Foundation [GA21-15958L] and the Swiss National Science Foundation [205321L_197275/1].

Keywords: Rhodospirillum rubrum, pigments, carotenoids, FTIR, RAMAN, spectrophotometry

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Computational study on amyloidoses through disease-protein-drug interaction network analysis

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The vast amount of high throughput data from genomics, proteomics and metabolomics experiments has brought on the era of Systems Biology. In this work, Network Biology is used for data integration and analysis of protein, drug, and disease interactions. The aim of this study is the investigation of amyloidoses, which is a group of rare diseases caused by the deposition of amyloid fibrils in organs and tissues. All known amyloidoses and clinical conditions associated with amyloidosis were extracted from AmyCo, along with the precursor proteins and the co-deposited proteins found in amyloid deposits. Protein-protein interactions were collected from STRING database. In addition, protein-drug interactions and disease-drug associations were obtained from DRUGBANK and UniProt. Finally, we constructed a heterogenous network that contains diseases, proteins, and drugs. Subnetworks of each amyloidosis and precursor proteins were studied in detail to identify promising drugs, not previously investigated according to literature. Experimental studies of these drugs are proposed, while further network analysis could give rise to new common pathways between these diseases.

Keywords: amyloidoses, disease-protein-drug interactions, network analysis

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qPCR identification of selected probiotic bacteria in fermented white cabbage juice

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The juice made from fermented cabbage is a traditional drink frequently consumed as a source of probiotic bacteria. We investigated cabbage juice as a natural substrate for the growth of probiotic cultures before and after pasteurisation using molecular methods. According to the literature, four naturally occurring probiotic organisms in cabbage juice were selected (L. acidophilus, L. casei, L. delbruckii subsp. bulgaricus, and L. plantarum), and their occurrence was subsequently monitored in the samples using real-time PCR. The PCR products were detected on an agarose gel.

Firstly, DNA amplification was verified using specific primers for the genus Lactobacillus and individual species of probiotic microorganisms. The genus Lactobacillus's presence was confirmed in fresh and pasteurized cabbage juice. Due to the decrease of the probiotic viability after pasteurization, the cabbage juice was enriched with the addition of probiotics. Their presence was monitored using cultivation methods and qPCR for two weeks after inoculation. The presence of individual selected species in conditions of enriched cabbage juice was confirmed for all samples in both time intervals, although with a low percentage of probiotic viability.

Acknowledgments
This work was also supported by the project at FCH BUT no. FCH-S-22-7961.

Keywords: cabbage juice, probiotics, qPCR, primers, amplification

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Effects of hypoxic preconditioning on neurovascular unit components and cognitive functions in rat's brain

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Hypoxia induces cerebral changes including cognitive impairment. Our aim was to assess the effects of hypoxia and the neuroprotective effects of hypoxic preconditioning at the cerebral level and its role in maintaining cognitive functions. Wistar rats (n=15) were equally divided into 3 groups: sham group (21% O2), hypoxic group (3h, 7% O2) and hypoxic preconditioned group (5 days of 6 min 7% alternating with 6 min 21% O2, in 5 cycles, 1 day of 1 h, 7% O2). The effects on cognitive functions (short memory) were evaluated using the Y–maze behavior test. Finally, all rats were sacrificed and brains were collected. We performed brain histopathological analysis through light microscopy and immunofluorescence staining using endothelial (CD31), pericyte (PDGFRβ), astrocyte (GFAP), Myelin and vasculature integrity (Notch3) markers. The preconditionated rats showed no change in cognitive performance following hypoxia compared to the hypoxic group. Hypoxic rats presented histopathological changes in neurons and oligodendrocytes, and also considerably higher mesencephalon vascular capillary density and diameter. The CD31/PDGFRβ / GFAP, Myelin and Notch3 expression in hypoxic rats differs significantly compared to sham and preconditioned rats. In conclusion, hypoxia induced changes in the brain cytoarchitecture and cognitive functions, while hypoxic preconditioning induced neuroprotective effects.

Keywords: Hypoxic preconditioning, neurovascular unit, cognitive functions

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Molecular cloning and heterologous expression of a recombinant SARS-CoV-2 accessory protein

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An unexpected pandemic of the new disease COVID-19 (coronavirus disease 2019) caused by a Coronaviridae family member, SARS-CoV-2, has affected the population globally. In order to develop successful prophylactic and therapeutic strategies against SARS-CoV-2 and other coronaviruses, it is of paramount importance to answer questions concerning the host-virus interaction. Interestingly, the accessory proteins of coronaviruses have been less studied than the other viral proteins but they appear to be crucial immunopathological factors. ORF3a, the largest accessory protein, is a multifunctional protein that has been reported to be involved in virus release and host immunomodulation. To this end, the aims of our study were the molecular cloning and heterologous expression of the cytoplasmic part of SARS-Cov-2 ORF3a. The ORF3a gene was initially isolated with cDNA synthesis and PCR from COVID-19 positive RNA samples and cloned into the expression vector pET-28a(+) by Restriction Enzyme Cloning. Furthermore, the recombinant His-tagged protein was expressed in E.coli Rosetta(DE3)pLysS bacterial cells, by induction with IPTG. Several conditions of induction and lysis were examined for optimizing protein expression. Our future goal is the use of the purified recombinant polypeptide of ORF3a in in-house immunoassays in order to evaluate its immunodiagnostic capacity.

Keywords: SARS-CoV-2, coronavirus, heterologous expression, recombinant protein, ORF3a

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Protein extracted from By-Products for valorisation in Circular Economy

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The proteinic by-products from leather industry (based on collagen) are suitable for many industrial applications: adhesives, surfactants, auxiliaries for leather processing, biodegradable packaging etc. but are also generous energy sources for biostimulation and organic fertilization of plants and soil enrichment. Their processing and introduction into the economy in the context of the green deal, produces a significant reduction of the environmental impact. From analytical investigation (structural, textural, particle size etc.) it has been found that proteinic composites extracted from by-products contain considerable proportions of large size components, over 1000 nm, with film-forming properties and controlled biodegradability with delayed release of amino acids and oligopeptides. Also, the proteinic composites contain sufficient proportions of small and medium size components, 1-100 nm and 100-1000 nm, specific for free amino acids and oligopeptides. In industrial applications, large molecular compounds induce the adhesive and film-forming properties while small and medium-sized compounds are associated with the surfactant properties. In agriculture applications, the large size proteinic components ensure the delayed release of amino acids and oligopeptides for a gradual feeding of the plants in various vegetation stages, once the small molecules capable to penetrate cell membranes have immediately acted with systemic effects on biostimulation and protection.

Keywords: by-product, collagen, amino acid, adhesive, biostimulation

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Aubrac cattle, a new trend for beef cattle breeders

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The Aubrac cattle breed are found in Aveyron-Lozère, France. Their development started during the 1600's at the Benedictine Abbey of Aubrac in the south of France. They are used primarily for meat production although, historically, they were also used as draft animals and for milk. The cows can reach a maximum weight of about 800-900 kg, and a bulls can weigh up to 1,200 kg with an average daily gain of about 1300 grams/day. Because the Aubrac breed is so low-maintenance and cost-effective, they can do well on small-scale farms. One great thing about the Aubrac breed is that they can eat low-cost, rough forage such as grass and hay and still produce quality meat. According, Domestic Animals Diversity Information System (DadIs/FAO), the populations of Aubrac cattle varies from country to country, respectively: Romania (808), Austria (1000), Bulgaria (59), Canada (7), Croatia (180), France (471974), Germany (885), Ireland (4700), Lithuania (12357), Luxembourg (111), Hungary (349), United Kingdom (50). The Aubrac cattle breed is a popular one due to the excellent quality of meat they produce, famous for the special aroma and tenderness of the meat, having a grade high marbling and a great taste and a very good bone/meat ratio.

Keywords: beef, performance, populations, quality

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Design of cellulose based micro- and nanostructures for encapsulation and release of curcumin

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Poor aqueous solubility and bioavailability of interesting bioactive compounds is a challenge in the development of bioactive formulations. In this work, microcrystalline cellulose (MCC), cellulose nanocrystals (CNC) and cellulose nanofibers (CNF) were investigated as carriers for the delivery of curcumin, a model liposoluble compound. The mixture with Tween 40 and chitosan, as well as the functionalization with the cationic surfactant CTAB or by TEMPO-mediated oxidation were also studied. FT-IR and PXRD analyses were performed as structural characterizations, while the delivery systems were evaluated in terms of entrapment efficiency, loading capacity, yield, surface charge, particle size and polydispersity. The release profile was evaluated in conditions that mimic the gastrointestinal fluids, namely pH, temperature and time of exposure in simulated gastric fluid (SGF) and intestinal fluid (SIF). The results showed that cellulose nanostructures are more efficient in curcumin binding than MCC. Modification with CTAB and coupling with chitosan resulted in higher binding efficiencies (90-99%) and a faster release of curcumin. CNC-Chitosan released 50% of curcumin after 1 h in SGF, while CNC-CTAB released only 20% in SGF (after 2 h), but up to 47% in SIF (after 8 h), which may be particularly interesting for intestinal delivery of bioactive compounds.

Keywords: delivery systems, curcumin, nanocellulose, release profile

[PP-048] DOI: 10.2478/ebtj-2023-0004

Gold-pluronic nanocomposites associated with chloro-aluminum phtalocyanine in pregnant with breast cancer

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Breast cancer is a multifactorial neoplasm, and tumor heterogeneity is one of the biggest challenges during treatment. In pregnant women, for more rare as it may be, it is defined as breast cancer diagnosed three months before abortion, during pregnancy or one year after delivery. The treatment of pregnant women with breast cancer presents a unique and challenging scenario, requiring a balance between the risk-benefit for both the mother and the fetus. The present study is using gold-pluronic nanoparticles (AuNP-AlClPc) associated with a photosensitizer chloro-aluminum phtalocyanine. AuNPs can act as both photothermal agents and FS carriers, allowing the simultaneous use of TFD and TFT. AuNP-mediated TFT-PDT combination therapies conjugated with AlClPc may be more effective and low-risk maternal-fetal alternatives for pregnant women with breast cancer. Aiming at future pre-clinical applications of these therapies, aspects related to biodistribution and biocompatibility Photodynamic Therapy is a non-invasive therapeutic approach based on photoinduced that involves the generation of reactive oxygen species (ROS) through the simultaneous use of light, molecular oxygen and a photosensitizing agent. Photothermal Therapy is not dependent on local oxygen and can be used to treat cancers that normally do not respond to PDT treatment.

Keywords: Nanobiotechnology, Cancer, Nanoparticles, Breast cancer

[PP-049] DOI: 10.2478/ebtj-2023-0004

Complex characterization of indigenous yeast strains isolated from wine wort, for applications in biomedicine and food industry

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Replacement of laboratory microbial strains with strains isolated from different ecological niches, harboring naturally acquired phenotypic characteristics, might represent an efficient alternative for improvement of processes from food industry, where there is an increasing emphasis on preserving cultural identity and capitalizing artisanal food products. The present study deals with the characterization of four new yeast strains from spontaneously fermented wine wort (Ilfov County, Romania). Taxonomical identification (conventional tests, RFLP-PCR, sequencing of ITS1-5.8SrDNA-ITS2 region) revealed that the strains belong to Torulaspora, Saccharomyces and Pichia genera. All the strains were able to develop under thermal, osmotic or ionic stress conditions, and tested negative for virulence and pathogenicity factors. Esterase production resulted in release of butyric acid, a prebiotic compound. The yeast strains showed good capacity to inhibit the growth of human pathogens (Candida sp., Escherichia coli and Pseudomonas aeruginosa), showed limited activity against probiotic microbial strains and impaired the mycelial growth of filamentous fungi mostly belonging to Aspergillus flavus species. In conclusion, the present work allowed the complex characterization of four new indigenous yeast strains with important antimicrobial properties that recommend them as promising microbial resources for applications in biomedicine and food industry.

Keywords: yeasts, wine wort, identification, antimicrobial activity, esterase, biomedicine

[PP-050] DOI: 10.2478/ebtj-2023-0004

The significance of the BRCA2 c.9934A>G variant is really unknown?A series of patients with BRCA2 c.9934A>G variant among Çanakkale patients

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The BRCA2 c.9934A>G(p.I3312V) (NM_000059.4) variant is classified as "Variant-of-Uncertain-Significance(VOUS)" according to ACMG criteria and found extremely low frequency in Gnomad population databases. In this case series, we aimed to raise the question that this variant may be pathogenic. After DNA isolation from peripheral blood, all exonic, exon-intron junctions of BRCA2 were analyzed using the inherited cancer NGS panel method in 519 patients with cancer and/or familial cancer history. 6 patients had the BRCA2 c.9934A>G variant in a heterozygous state. Patient 1-3, had breast cancer (20-47-51 years-old), Patient 4, 42-years-old ovarian cancer, Patient 5 and 6, have a family history of pancreas and breast cancer respectively (40-56 years- old). BRCA2 is a human tumor suppressor gene involved in the repair of chromosomal damage. Therefore, its pathogenic mutations confer an increased risk of cancers such as breast, ovarian, pancreas, etc. Our data revealed the c.9934A>G variant in the BRCA2 gene in 6 patients. Detection of this variant only in patients with personal and/or family history of BRCA2-related-cancers seems far from coincidental. We think that this variant may be pathogenic due to the clinical conditions and strong family histories of the patients.Larger cohorts would be useful to elucidate the pathogenicity of this variant.

Keywords: BRCA2, Cancer, VOUS

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A novel BRPF1 variant in a family with intellectual disability and dysmorphic face, from Çanakkale

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Intellectual developmental disorder with dysmorphic facies and ptosis(IDDDFP) is an autosomal dominant disorder characterized by delayed psychomotor development, intellectual disability(ID), and dysmorphic facial features caused by pathogenic mutations in the Bromodomain- and PHD Finger-Containing Protein(BRPF1) gene. Here we present a family with a pathogenic mutation in BRPF1. A 1-year-old male patient was referred to our clinic because of dysmorphic face and developmental delay. Microcephaly, micro-retrognathia, ptosis, pes equinovarus, and failure to thrive were present. The proband's mother was 21-years-old with a dysmorphic face, ID, and short stature. She has retrognathia, ptosis, small ear. The proband's sister is 2-months-old and has micro-retrognathia, broad nasal root, failure to thrive, and microcephaly. The proband's father was 38 years old and had no additional signs of ID.We performed whole-exome-sequencing(WES) analysis for the proband and his family. In WES analysis, we detected a heterozygous pathogenic novel variant c.1433G>A;p.W478*(NM_004634.3) on exon3 of BRPF1 in the proband, his mother, and sister. A total of 42 cases of BRPF1-associated syndrome have been reported so far. This syndrome is extremely rare and has not been reported in Turkey. Additionally, this report emphasizes the importance of advanced genetic techniques such as WES, in patients with ID, and dysmorphic facial features.

Keywords: BRPF1, dysmorphic facial features, IDDDFP

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Determination of carrier frequency of SMN1 gene intragenic mutations: Case series

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Introduction
SMA is an autosomal recessive lethal disease, characterized by degeneration of the anterior horn cells of the spinal cord, leading to symmetrical muscle weakness and atrophy. The disease is detecteble with CNV analyses (95%-98%) and sequence analysis (2%-5%). Carrier frequency of SMN1 deletion is 1/40-1/60 in Europian countries while there is no point mutation carrier frequency. We aim to present SMN1 point mutation frequency in our 500 WES datas in this Case series.

Method
We have performed the whole exome sequencing(WES) analysis by using the xGenExomeResearch Panel v2 kit. Qiagene Clinical Insight Interpret and IGV used for analysis.

Results and Discussion
We analysed 500 person's SMN1 gene. We detected one in-frame point mutation from 500 WES datas which is c.842G>C; p.R281T in exon 8 (NM_000344.4)(Varsome, ClinVar, QCI and Franklin evaluates as variant of uncertain significance). Exonic point mutation frequency is detected 1/500 in our cohort. In vitro studies have shown that this variant does not affect the function of SMN1 gene product but there are not enough studies in literature. And for the future, more extensive cohort studies can show us importance of intragenic mutations in SMN1.

Keywords: SMN1, SMA, Intragenic Mutation

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Heavy Metals in Soils from the Border Region Romania-Serbia

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The aim of this study was to establish a system for monitoring the heavy metal content of soils in the border area of Romania with Serbia, and the results were communicated to local and regional authorities and residents in the study area. The sources of soil contamination with heavy metals are varied, in general they are anthropogenic: mining tailings, industrial waste, gasoline, fertilizers, various pesticides, but some soils may have a native load with these metals, due to the rocks on which they formed. Soil samples were taken from 32 localities in the border area with Serbia, on three depths: 20 cm, 40 cm and 60 cm. The following types of soils were identified in the researched area: Fluvisols, Chernozems, Phaeozems, Eutric Cambisols, Luvisols, Vertisols, Stagnosols, Gleysols, Solonetz, Anthrosols, each with its distinct properties. The heavy metals analyzed were: Fe, Mn, Zn, Cu, Cd, Cr, Pb, Ni.

Keywords: soil, heavy metals, monitoring

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Yeast Killer Toxins For Biomedical Applications Against Human Pathogenic Candida Strains

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Killer yeasts synthesize extracellular toxins inhibiting the growth of other yeasts, including Candida pathogenic cells, without risks for human health. The strains Saccharomyces cerevisiae CMGB59 and Kluyveromyces marxianus CMGB230 were tested for activity of the killer toxins in presence of five Candida strains from vulvo-vaginal infections. Positive results were determined against Candida famata CMGB-M, Candida famata CMGB-Y14 and Candida utilis CMGB-15. The [15X] concentrated toxins and crude extracts were tested at different values of pH (4.4, 6.2) and temperature (28°C, 37°C), with best activity against C. famata CMGB-Y14 after 7 days of incubation at 37°C. The growth of C. famata CMGB-Y14 was strongly reduced (81.61% after 24 hours) by S. cerevisiae CMGB59 concentrated toxin (pH = 4.4), while the K. marxianus CMGB230 concentrated toxin (pH = 4.4) had the highest antiadherence activity (38.6%) against C. famata CMGB-Y14 biofilm formation. Both concentrated toxins (pH = 4.4) had high effect on the development of Candida pseudohyphae and production of lipases, gelatinase, caseinase and siderophores, with no influence on hemolysine and amylase synthesis. Further investigations will focus on the mechanism of action of the two killer toxins, for the development of new biomedical agents.

Keywords: yeasts, killer toxins, Candida, antimicrobial, antiadherence, biomedicine

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In vitro antimicrobial activity testing of 9 essential oils for application in the meat industry

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The efficiency of essential oils (EOs) was noted since ancient times. The research is based on testing the antimicrobial effects of 9 EOs (Cinnamomum camphora, Thymus vulgaris, Laurus nobilis, Pimpinella anisum, Mentha piperita, Boswelia sacra, Foeniculum vulgare, Citrus sinensis, Melaleuca alternifolia) on pathogens relevant to the food industry. For the study conduct, all tests were performed in triplicate, with positive and negative control. Using the Agar Well Difussion method, for E. Coli the highest inhibition zones were observed in EOs obtained from Cinamomum camphora (20.67±2.52), Thymus vulgaris (20.33±1.53) and Melaleuca alternifolia (17±1). For Staphylococcus aureus, EOs from Citrus sinensis (20.67±1.15), Thymys vulgaris (20.33±0.58), Cinamomum camphora (19.67±1.53) were the most efficient. For Enterococcus faecalis, Thymus vulgaris (21±1), Melaleuca alternifolia (16.33±1.53), Boswelia sacra (15.67±2.08) had antimicrobial effect. EOs from Thymus vulgaris (11±1), Pimpinella anisum (11.33±1.53), Citrus sinensis (8.67±1.53), were effective against Salmonella typhymurium, and those from Boswelia sacra (23.67±1.53), Thymus vulgaris (20.67±1.53), Mentha piperita (8±1) were effective against Listeria monocytogenes. The EOs with the highest overall efficiency are those obtained from Thymus vulgaris, Melaleuca alternifolia, Cinnamomum camphora, Citrus sinensis and Boswellia sacra. The results were concludent in order to select the EOs with the most promising antimicrobial effects for further studies.

Keywords: essential oils, meat industry, antimicrobial

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Overexpression of the oak dehydrin gene alleviates salinity stress in transgenic tobacco

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We aimed to study the effect of an overexpression of the oak dehydrin gene (AY60770) in transgenic tobacco plants in alleviation of salinity stress. Transgenic tobacco plants were prepared by introducing of the expression cassette consisting of the oak dehydrin gene (Dhn3) driven by the constitutive double dCaMV35S promoter, via Agrobacterium-mediated transformation. The seeds of transgenic plants overexpressing Dhn3 gene and the seeds of non-transgenic (control) plants were germinated on the media supplemented with 0 mM and 100 mM NaCl. After 21 days of growth, seedlings were analysed for the content of photosynthetic pigments and proline, as stress parameters. We found that the applied stress had no significant effect on the concentration of photosynthetic pigments in transgenic seedlings overexpressing oak dehydrin gene while in the stressed non-transgenic seedlings the concentration of photosynthetic pigments was significantly decreased. The proline content was significantly increased in the stressed seedlings over control non-stressed seedlings in both transgenic and non-transgenic lines. A more pronounce increase was observed in Dhn3 overexpressing seedlings.

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Keywords: dehydrin, Quercus robur L., transgenic tobacco, salinity

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The Content of Metals and Metalloids in Transboundery Surface and Groundwaters, in the Crossborder Romania – Serbia Banat Plain

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The purpose of this study, part of the Interreg IPA CBC Romania - Serbia Program, RORS project - 279 "Cross-border network for education and research of natural resources", was to monitor the content of metals and metalloids not constantly monitored by the authorities, and in concentrations exceeding the maximum permitted limits imposed by the EU-DWD, they have harmful effects on living organisms. It were established 24 sampling points (21-surface waters and 3-groundwater), considered representative for the evaluation of the quality of transboundery waters in Romania - Serbia Cross-border Area. The samples were collected monthly for 16 months, and by ICP-MS technique the following elements in maximum concentrations (µg·L-1) were determined: Ba 90,166; Pb 0,799; Sr 329,718; Zn 151,587; Ni 5,472; Cu 9,007; Cr 5,136; Al 118,223; B 475,038. The water samples represent boreholes used for drinking water supply were noted by a high content in Ba (90,166), Sr (310,109), Zn (151,187) and B (106,183, respectively 114,645). These high values have natural causes, due to the solubilization of the parent rocks. Comparing the values obtained with the limits imposed by EU-DWD 2021 and international legislation, exceedances were observed in the case of Sr (maximum allowance limit: 4 µg · L-1).

Keywords: metals, metalloids, pollution, transboundary waters

[PP-058] DOI: 10.2478/ebtj-2023-0004

Influence of Ultrasound Pretreatment on Microbiological Quality of Convective and Vacuum Dried Butternut Squash (Cucurbita moschata)

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Ultrasound pretreatment of vegetables has been a hot topic recently and has shown promise in significantly reducing overall processing time. The objective of this study was to investigate the effects of high-intensity ultrasound treatment with different amplitudes and treatment times using an indirect and direct ultrasound device on the microbiological safety of butternut squash by monitoring microbiological growth during 28 days of storage at room temperature. Classical microbiological methods of cultivation on selective media were used to determine the presence of microorganisms. The results showed that the shelf life of the squash was better with the combination of pretreatment and drying than with drying and/or pretreatment alone. All treatment combinations were microbiologically safe after 28 days. The best results were obtained with samples treated with ultrasound at an amplitude of 60 % for 6 minutes before vacuum drying, and no microorganisms were detected in any of the samples.

Keywords: ultrasound, drying, butternut squash, shelf life

[PP-059] DOI: 10.2478/ebtj-2023-0004

Plant protection bacteria increase food safety by inhibiting human pathogen contamination of vegetables

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Herbs, vegetables and fruits, especially from organic agriculture, are highly exposed to animal and human pathogens contamination. Microbial antagonists can be used to prevent this risk to occur. Among plant beneficial microorganisms there are promising biocontrol strains. Considering this, the aim of this study is to reveal several plant beneficial bacterial strains that can be used to promote plant health and nutrition, while reducing the prevalence of human and animal pathogens. Tested beneficial strains are adapted to live as endophytes, thus being able to express an intimate relation with their plant hosts. Within this study we analysed the inhibitory activity of seven biocontrol strains against 19 pathogenic bacterial strains. Some of the studies strains revealed antibacterial activity against a wide spectrum of pathogens, such as: Bacillus cereus, Escherichia coli, Listeria innocua, L. ivanovii, L. monocytogenes, Rhodococcus equi, Salmonella typhimurium, Staphylococcus epidermidis and Streptococcus pyogenes. Due to their antagonistic activity, the beneficial strains were studied through molecular techniques to reveal their functional genes involved in antibacterial compounds synthesis. Genes encoding for iturin A, surfactin, fengicin and bacillaene were found in these biocontrol strains. Therefore, we could consider such beneficial strains as promising candidates for plant protection and human safety.

Keywords: biocontrol endophytes, antagonism, pathogenic bacteria, functional genes

[PP-060] DOI: 10.2478/ebtj-2023-0004

Extraction of phenolic compounds from grape seeds and their characterization

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Polyphenolic compounds present in grape seeds have a strong antioxidant and antimicrobial effect. The optimal conditions for the production of Pinot Noir red grape seed extracts have been determined. Three types of organic solvents were used - acetone, methanol and ethanol. It was found that the safest extract, good extraction yield and total phenolic content was achieved using a mixture of 70% ethanol and 30% deionized water. The impact of different techniques for the isolation and purification of antioxidants from grape seeds was investigated. Two conventional methods (with shaker and magnetic stirrer) and two non-conventional methods (with ultrasonic bath and microwave oven) were used. It was found that the extraction of Pinot Noir red grape extracts using a magnetic stirrer provides good efficiency (extraction yield 18% and total phenolic content - 444.87 mg galic acid/g dry weight extract) and the process time is relatively short – 3 hours. The total antioxidant parameters of seed extracts of varieties of three red grapes (Cabernet Sauvignon, Marselan, and Pinot Noir) and one white grape (Tamianka) obtained with the optimal extraction conditions were determined. The obtained total phenolic contents, total flavonoids, anthocyanin pigments and vitamin C for the four extracts were compared.

Keywords: antioxidants, grape seeds, extraction, antioxidant parameters

[PP-061] DOI: 10.2478/ebtj-2023-0004

Loop-mediated isothermal amplification (LAMP) for detection of human and plant pathogens using Invitrogen™ SuperScript™ IV RT-LAMP Master Mix (2X)

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Rapid and accurate detection of viruses, bacteria and fungi, is essential for carrying out disease prevention, diagnostics and surveillance. Loop-mediated isothermal amplification (LAMP) is a promising technology for rapid and sensitive identification of a selected target that can be implemented in medicine, food industries and agriculture. LAMP is a low-cost technology allowing for simple reaction preparation and target nucleic acid amplification in 5-30 minutes under isothermal conditions of 60-65°C using a simple thermoblock. Here, we demonstrate the use of Invitrogen™ SuperScript™ IV RT-LAMP Master Mix and custom designed primer sets for rapid detection of human and plant pathogens including SARS-CoV-2, human adenovirus, Staphylococcus aureus, potato spindle tuber viroid, Fusarium graminearum, Botrytis cinerea, and Phytophthora cactorum. Demonstrated results are obtained via real-time fluorescence detection using Invitrogen™ SYTO™ 9 stain as well as "naked eye" colour-change endpoint detection method using Invitrogen™ SYBR™ Green I stain. Finally, we report that Invitrogen™ SuperScript™ IV RT-LAMP Master Mix can be implemented during the development of highly accurate, sensitive and rapid LAMP assays for the detection of nucleic acids from human and plant pathogens.

Keywords: LAMP, reverse transcription, visual detection, plant pathogens, human pathogens

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The performance of fat swine according to the welfare conditions offered by the production system

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Research conducted in the conditions of Satu-Mare County, Romania, highlights the production performance of fat pigs in different maintenance systems, each system involving obtaining a certain type of meat, production costs, production price, investment efforts, production indicators and different economic results. Due to the high demand for meat on the regional and local market, swine farms from the researched area must have the rapid ability to adapt to the needs and consumption preferences of consumers. These integrated farms can only meet these demands only if they have implemented production management which ensures the best welfare conditions for fattening pigs and have high-performance operating systems that meet these requirements. In order to improve the welfare conditions of fattening pigs according to the quantity and quality of meat required by the market, farms must be able to improve their systems and operating conditions to ensure welfare: maintenance, feeding, watering and control of microclimate factors and to specialize human resources to meet these demands.

Keywords: swine, production systems, meat, welfare

[PP-063] DOI: 10.2478/ebtj-2023-0004

Development of an aptasensor for rapid and specific detection of Escherichia coli in water and milk samples

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Escherichia coli is part of the normal flora of the gastrointestinal tract and is a biological indicator of fecal pollution in water and foods. Some strains are known as agents causing food and

water poisoning. In 2019 219,000 human zoonoses (WHO) with significant health and economic impacts were caused by E. coli which can also be considered as an indicator in antimicrobial resistance (AMR) surveillance systems for environment, food and clinical data. Although several methods have been developed for E. coli detection and quantification rapid methods are still missing, especially for liquid samples. Biosensors have gained interest for their specificity, rapidity and ease to use in various fields as they can utilize ssDNA probes and aptamers as bioreceptors providing high specificity and sensitivity. In this work an aptasensor for the detection of E. coli in water and milk samples has been constructed and compared with classical methods. Methods for the aptamer immobilization on the electrode and time for cell detection were optimized reaching a sensitivity of 10^2 cells/mL in 30 min. Moreover, AMR was evaluated for E. coli isolates to obtain a complete picture of the spread of resistant E. coli among water and foods.

Keywords: aptasensor, Escherichia coli, antibiotic resistance, water, milk

[PP-064] DOI: 10.2478/ebtj-2023-0004

Red Grape Peel Extract – A Promising Functional Ingredient for Improving the Bioactivity and Phytochemical Profile of Beer

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The red grape peels represent an important source of bioactive compounds, with many potential health benefits, that can be used as natural antioxidants and colorants in food industry. In the present study the antioxidant-rich red grape (Băbească neagră) peels extract (GSE) was used to increase the biological value of beer. The beer supplemented with 1-10 g GSE/L was characterized in terms of total phenolic content (TPC), total flavonoids content (TFC), total monomeric anthocyanins content (TMA) and radical scavenging activity. Beer supplementation with GSE resulted in significant increase of TPC and TFC from 3.337 to 4.352 mg GAE/mL, and from 0.837 to 1.474 mg CE/mL, respectively. Similarly, the TMA of beer samples gradually increased from 0.007 to 0.030 mg C3G/mL with increasing the addition level of GSE. Since GSE is rich in anthocyanin pigments, beer acquired a reddish color. As expected, the radical scavenging assay indicated the linear increase of the antioxidant capacity of beer with the increase of GSE. The incorporation of GSE resulted in increased level of bioactive compounds, which suggest the possibility of using the GSE as promising functional ingredient for brewing specialty beers.

Keywords: beer, grape peels, phenolics, anthocyanins, antioxidant capacity

[PP-065] DOI: 10.2478/ebtj-2023-0004

Probiotics modulate the immune response and tight junction strength in an intestinal epithelial barrier in vitro model

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Healthy gut microbiota is essential for human well-being. However, pathogens and pro-inflammatory stimuli can cause an imbalance in the gut microbiota, an increase in intestinal permeability and systemic inflammation. The present study aims to (i) evaluate the efficacy of a multi-strain probiotic formulation (Serobioma®, Bromatech s.r.l., Italy), in the prevention of lipopolysaccharide (LPS)-induced inflammatory damage, (ii) study the role of mast cells in gut inflammation and (iii) explore underlying molecular mechanisms. The experimental in vitro model consists of a monolayer of Caco-2 and/or HT-29 intestinal human cells cultured on a semi-porous membrane of a transwell insert, which is interfaced with a human mast cell line in the basolateral chamber. Transepithelial Electrical Resistance was used to evaluate the intestinal epithelial barrier integrity at baseline and after LPS/Serobioma exposure. Using Real-Time PCR, variations in Tight Junction Protein (TJP) gene expression in intestinal cells were examined. Mast cell cytokine release was quantified using the ELISA assay. The results show that Serobioma prevents LPS-generated intestinal epithelial barrier dysfunction by modulating the expression of TJP genes in intestinal cells and the release of IL-6 from mast cells. These findings suggest that Serobioma could be a valuable support for the treatment of gut inflammation.

Keywords: gut inflammation, probiotics, tight junction, IL-6, mast cells, immunomodulation

[PP-066] DOI: 10.2478/ebtj-2023-0004

Forecasting and planning sows production to obtain quality carcasses

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Taking in consideration the new sustainable alternatives for obtaining quality carcasses by forecasting and planning the production of animal protein, it is necessary to orient swine production towards obtaining meat at low prices, for the benefit of promoting higher incomes for farmers through subsidies, covering in the same time the supplementary costs of animal welfare raised in sustainable production systems and environmental risk. Growing needs for swine meat generates the increase of

production and consumption, but although production systems offer a wide range of benefits, including employment services, we believe that production forecasting and planning to meet market demands must be find solutions to obtain the necessary quantity of quality meat without an uncontrolled increase in the number of sows which calls into question the sustainability of production due to increased pressure on water, energy and soil and increased greenhouse gas and waste emissions. Maintaining a constant flow of fattening swine to the slaughterhouse and constant sales of quality meat, integrated farm management must monitor at all times the electronic evolution of the herds of the three groups of sows used for breeding, for consistency of production and ensuring the necessary swine youth for fattening

Keywords: production system, sows, management, carcasses, quality

[PP-067] DOI: 10.2478/ebtj-2023-0004

Technological methods to improve the quality of fat from swine carcasses

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In order to obtain carcasses with low amounts of fat and of quality, it is necessary for the farm management to ensure adequate maintenance shelters and fodder suitable for the intended purpose, sufficient quantities of water and reliable technologies which can be adapted to the requirements of the meat market. Obtaining quality fat carcasses requires reducing the sensitivity of fat pigs to farming technologies, maintaining animal health and their biosecurity by improving production indicators, ensuring the welfare of fat pigs through technological conditions that allow them to perform, the use of healthy commercial pigs with high capacity to adapt to exploitation, health control through a veterinary sanitary management of prevention and ensuring the biosecurity of animals and fattening farms. Maintaining the quality of swine meat is critical, and the quality of feed influences the quality of meat and fats whose quality is defined in terms of physical and nutritional characteristics but major problems regarding the quality of meat fats are soft fats, unusual odors and the impact of fat composition on meat consumers. Reducing the soft fat from swine carcasses is a major concern for meat producers and processors as it can cause problems in meat processing.

Keywords: swine, technology, carcass, quality, fats

[PP-068] DOI: 10.2478/ebtj-2023-0004

Tyr analogues of BIM-23052 - synthesis, biological activity and mathematical predictions

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Somatostatin (SST) analogues are promising alternative of conventional chemotherapeutics against cancer in a medicinal practice. D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH2 (BIM 23052) is a linear analogue of SST with established in vitro GH-inhibitory activity in nM concentrations and high affinity to several SST receptors [1]. Lipophilicity is a key factor in the ability of molecules to cross cell membranes and perform their biological function. Herein, we report synthesis and anticancer activity of a series of BIM-23052 where Phe residues are replaced by Tyr. The modifications in the primary structure of BIM-23052 are done in different position of the parent molecule and both L- and D-Tyr are used. In addition, some calculations on the log P values of all molecules as well as docking calculations according to the SST receptors are done. Finally, some structure-activity relationships will be discussed.

[1] Coy DH, Murphy WA (1997) Linear somatostatin analogs. US Patent, Patent Number 5633263, 27 May 1997. http://www.lens.org/images/patent/US/5633263/A/US_5633263_A.pdf

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Keywords: BIM-23052, Tyr analogues, anticancer activity, docking calculation, log P calculation

[PP-069] DOI: 10.2478/ebtj-2023-0004

Bioconjugates of BIM-23052 analogues with unnatural aminoacid - synthesis and biological study

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The specific activity of the so-called biologically active peptides is embedded in their primary structure. Introducing of unnatural amino acids in the peptide chain often influence positively pharmacodinamic and pharmacokinetic newly synthesized compounds. Our previous studies reveal that analogue of BIM-23052, a linear derivative of somatostatin, which contain unnatural amino acid aminoisobutanoic acid (Aib) and aminocylcohexanoic acid (Ac6c) in the structure, has very good antiproliferative activity against panel of tested tumor cell lines [1]. On the other hand peptides are often used as cargo for transportation of second pharmacophore as diagnostic tool, vectors for delivery at specific cells or tissues or to create molecules with two pharmacophors with synergic effect. Herein, we will present synthesis and biological investigations of bioconjugates of analogue of BIM-23052 D-Phe-Phe-Phe-D-Trp-Lys-Aib-Phe-Thr-NH2 with second pharmacophore with well-established anticancer properties such as caffeic acid, 1,8-naphtalimide and tripeptide Arg-Gly-Asp.

[1] E.D. Naydenova, D. Wesselinova, S.Ts. Staykova, D.L. Danalev, T.A. Dzimbova, Synthesis, in vitro biological activity and docking of new analogs of BIM-23052 containing unnatural amino acids, Amino acids, 2019, 51 (9):1247–1257.

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Keywords: BIM-23052 analogues, unnatural amino acids, aminoisobutanic acid, aminocyclohexanoic acid

[PP-070] DOI: 10.2478/ebtj-2023-0004

Biocompatibility and antimicrobial behavior of silver doped SiO2/pectin hybrid materials

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In this study it was investigated the biocompatibility and antimicrobial behavior of silver doped hybrid materials containing SiO2 and citrus pectin. The varied silver concentrations (0 - 2.5 wt. %) affect the survival of L929 mouse fibroblast cells and the antimicrobial behavior of materials against Candida albicans 74. The results reveal that materials with containing 0.5 wt. % and 1 wt. % Ag showed biocompatibility as well as revealed low cytotoxicity, good cell adhesion and spreading and luck of apoptosis. The cytotoxicity of materials was influenced by the silver content and exposure time. The actin cytoskeleton organization also depends on the amount of Ag – the lower concentrations up to 1.5 wt. % caused the formation of actin stress fibers and filaments. It was established that the increase of silver concentrations leads to growth inhibition of C. albicans 74 with formation of clear zones around the materials. The silver doped SiO2/ pectin composite materials were proven to be efficient for use as in vivo implants in tissue engineering applications.

Acknowledgements: The work is a part of National Program "EUROPEAN SCIENTIFIC NETWORKS" of Ministry of Science and Education of Bulgaria, "Drug molecule" D01-278/05.10.2020.

Keywords: silica-pectin composites, silver, biocompatibility, antimicrobial behavior, cytotoxicity

[PP-071] DOI: 10.2478/ebtj-2023-0004

Synthesis, anticancer and antimicrobial activity of (KLAKLAK)2-NH2 analogues containing unnatural amino acid nor-Leu and conjugates with second pharmacophore

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(KLAKLAK)2 is an antimicrobial peptide with established anticancer properties as on internalization it causes mitochondrial swelling and destruction of the mitochondrial membrane leading to apoptosis [1]. Introducing of unnatural amino acids in the primary structures of peptides often influence positively pharmacodinamic and pharmacokinetic properties of obtained compounds. Our previous investigations reveal that replacement of natural amino acid Ala in the primary structure of (KLAKLAK)2 with unnatural analogue beta-Ala leads to good antiproliferative properties against panel of tested cell lines as well as good antimicrobial activity against model strains G+, G- microorganisms and fungi and also perfect hydrolytic stability at different pH values for 72 hours. Herein, we report synthesis, antiproliferative properties and antimicrobial activity of (KLAKLAK)2-NH2 analogues containing unnatural amino acid nor-Leu and conjugates with second pharmacophore with proven anticancer properties such as caffeic acid and 1,8-naphtalimide. Some structure-activity relationships will be also discussed.

[1] Thundimadathil, J. Cancer Treatment Using Peptides: Current Therapies and Future Prospects. J. Amino Acids 2012, 2012, 967347, doi:10.1155/2012/96734
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Keywords: (KLAKLAK)2 analogues, antimicrobial activity, biocongugates

[PP-072] DOI: 10.2478/ebtj-2023-0004

A novel homozygous mutation in CC2D1A gene: two case from a Turkish family in Canakkale

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CC2D1A gene is associated with autosomal ressesive non-syndromic intellectual disability (NS-ID) characterized by mental retardation, delayed psychomotor development, and speech abnormality. Here we report two siblings with homozygous CC2D1A gene mutation.

A 24-year-old female and a 15-year-old male siblings, whose parents were consanguineous, were referred to our outpatient clinic. Both patients had intellectual disability, epilepsy, autistic behavior and speech retardation. In physical examination the female patient had short stature, microcephaly, triangular face, microtia, beak nose, long filtrum and thin-narrow mouth. The patient had a medical history of seizures, but she had not had seizures for five years. The male patient had dysmorphic facial features such as a bulging nose, long face, and narrow forehead. The patients had a male and five female healthy siblings. We performed microarray analysis, thereafter whole exome sequencing (WES) analysis. Microarray analysis showed no deletion or duplication for both patients. We found homozygous novel CC2D1A c.1264C>T;p.Q422* (NM_016139.4) variant in WES analysis of the patients. We concert segregation analysis for parents. CC2D1A-related NS-ID has been reported rarely in the literature. It also associated with autism spectrum disorder and seizures. In addition, this report highlights the importance of advanced genetic techniques such as WES, in patients with ID.

Keywords: CC2D1A, non-syndromic intellectual disability, whole exome sequencing

[PP-073] DOI: 10.2478/ebtj-2023-0004

Improving meat processing methods obtained from intensively farmed swine

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The ability of swine farms to constantly produce meat and to adapt to the demand of the processing industry for the marketing of a certain quantity of meat of a certain type, depending on the preferences of consumers from the market represents an essential condition in swine raising and exploiting in integrated production. Obtaining good economic results in the swine meat industry involves, in addition to competitive individual production and improving meat processing methods, obtaining quality carcasses, high recovery yields at slaughter, different ways of cutting, distribution and recovery. Improving processing methods in case of the meat in carcasses distribution involves the implementation of measures to control stunning time, to obtain quality carcasses without blood stains, to extend the period after slaughter where the animal will remain hanging to collect blood and obtain carcasses well drained, by adjusting the speed of movement of the conveyor and evisceration will be done by cutting on the abdomen to avoid contamination, in order to obtain a carcass with good hygienic properties. In the case of meat marketed in sliced portions, the carcasses after refrigeration or freezing must be brought to the optimum storage temperature.

Keywords: swine, processing, carcasses, marketing

[PP-074] DOI: 10.2478/ebtj-2023-0004

Influence of mediators secreted by cancer-associated fibroblasts on genitourinary system cancer cells

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The role of cancer-associated fibroblasts (CAFs) in the cancer microenvironment is still being investigated and remains unclear. Molecules synthesized by fibroblasts found in the cancer environment may affect the progression of prostate and bladder cancer. We used Human Prostate Tumor-Associated Fibroblasts (HC-6223) to examine the influence of secreted factors on the bladder cancer cell line (HB-CLS-1) and prostate cancer cell line (DU145). For this purpose, we used conditioned media from HC-6223 cells. MTT test was used to measure cell viability and the BrdU test (synthetic thymidine analog that is incorporated into the DNA of dividing cells) was used to measure cell proliferation. HC-6223 conditioning medium (HC6223-CM) increased HB-CLS-1 proliferation after 48h and 72h respectively by 13% and 12% and viability respectively by 9% and 12% compared to control. Incubation of the DU145 cell line with HC6223-CM by 48h and 72h increased their viability respectively by 16% and 43% and their proliferation after 48h by 15% compared to control. Obtained results show that the mediators synthesized by CAFs interact with prostate and bladder cancer cells by increasing their viability and proliferation. The outcome indicates the need for more research on the tumor microenvironment and interactions between normal and cancer cells.

Keywords: bladder cancer, CAFs, cancer microenvironment,

prostate cancer

[PP-075] DOI: 10.2478/ebtj-2023-0004

Isolation, Purification and characterization of Non-Digestible Oligosaccharides (NDOs) from Manipuri Black Rice (Oryza sativa.L)

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Non-Digestible Oligosaccharides(NDOs)are the most common prebiotics which are low molecular weight carbohydrates with degree of polymerization (DP) 3-20, that are delivered intact to the large intestine. They facilitate prevention and treatment of lifestyle related disorders. In the present study, black rice oligosaccharides (BROs) were extracted at optimized process parameters. They were purified through charcoal-celite column and further by dialysis membrane (500 Da).The obtained black rice oligosaccharides proved to be non-digestible when subjected to gastric acidity, salivary and pancreatic amylases. The positive prebiotic score of BROs obtained in presence of different Lactobacilli strains is indicative of their prebiotic potential. Keeping in view the pharmacological importance of functional oligosaccharides and their proven efficacy over polysaccharides, BROs could serve as efficient prebiotics and can be further explored as functional food additives.

Keywords: Black rice, Characterization, Non-digestible oligosaccharides, Prebiotics, Functional food

[PP-076] DOI: 10.2478/ebtj-2023-0004

Isolation and characterization of faecal extracellular vesicles from mice and application for the study of helminth infections

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Soil-transmitted helminth infections are neglected tropical diseases, affecting over one billion humans worldwide, mostly marginalized people in low-income nations. The World Health Organization (WHO) advocates an integrated approach to controlling these illnesses, which includes proper sanitation or throughout vaccination. Extracellular vesicles (EVs) are lipid-bilayer bounded, non-replicative particles that are released by cells. They are classified into subtypes according to size and biogenesis, namely exosomes, microvesicles and apoptotic bodies. The microbiota is a complex biological system, the largest congregation of microorganisms forming a community live in the digestive tract. Microbiota-derived EVs in the intestinal track of infected individuals may be significant factors in host–helminth–microbiome crosstalk as their immunomodulatory payload interacts with the host. The aim of this work was to develop an appropriate workflow for the separation of EVs released by prokaryotic cells. A biochemical analysis of EVs, including purity determination, analysis by transmission electron microscopy for the visualisation of vesicles as well as the measurement of the concentration in each sample by nanotracking particle analysis and confirmation of EV-specific markers using western blot were performed. These results open new avenues for the study of microbiota EVs and their role in host-parasite-microbiota interactions.

Keywords: Extracellular vesicles, transmission electron microscopy, nanoparticle tracking analysis, western-blot, orthogonal biophysical separation, microbiota

[PP-077] DOI: 10.2478/ebtj-2023-0004

Fibrinolytic activity and application of protease Aspergillus candidus 305

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Cardiovascular diseases are the main cause of death in the world. Therefore, search and development of new therapeutic agents are significant. The application of micromycetes as producers of fibrinolytic agents has great potential. In this work, fibrinolytic activity of Aspergillus candidus 305 has been studied. It was found that the optimum conditions for growth and protease production were the highest at pH 6.0 and temperature 37°C. The maximum plasmin-like activity of A.candidus was observed after submerged cultivation for 96 hours, reaching 72,674 U/ml×10-3. Additionally, fibrinolytic activity was detected using fibrin plates (584,415 AU/ml). After isoelectric focusing a protease with pI 3,44 – 4,47 was detected. Isolated enzyme had high plasmin-like and thrombin-like activity determined with chromogenic peptide substrates – 38,86 E/ml and 44,254 E/ml, respectively. The molecular weight of the protease of A. candidus 305 - 35 kDa was determined by SDS-PAGE. Dot blot method and PAS-reaction showed that protease of A. candidus 305 is not glycosylated. The inhibitory analysis indicated that the protease belongs to serine class. Thus, represented data show the potential of A. candidus 305 and its application in thrombolytic therapy.

Keywords: proteases, plasmin-like activity, fibrinolytics, Aspergillus

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Effect of a biostimulant based on polyphenols and glycine betaine on the responses to salt stress in tomato

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Environmental abiotic stresses, exacerbated by climate change, have emerged as the main threats to agriculture in this century. Soil salinity, in particular, is one of the most damaging agents for the life cycle of plants. The present work evaluated the possible effect of a commercial biostimulant in improving salinity stress tolerance in tomato plants. The experimental approach consisted of a multivariate design including tomato plants, three levels of soil salinity and three doses of biostimulant (0 L/ha, 2 L/ha and 4 L/ha). The evaluation was carried out 30 days after the start of the treatments. The application of BALOX® biostimulant had a positive effect on plant growth under different soil salinity conditions, increasing plant fresh weight, protecting the root system, stimulating photosynthesis and partly inhibiting the uptake by the roots of toxic Na+ and Cl- ions. Growth in saline soils increased the stress affecting the plants, as shown by the accumulation of proline, the increase in malondialdehyde (MDA, an oxidative stress biomarker) contents, and the activation of antioxidant enzymes. Application of the biostimulant reduced the level of stress, as indicated by a relative decrease of these biochemical markers.

Keywords: Biostimulants, climate change, salt stress, oxidative stress, ion transport

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Characterisation of enzyme profiles of lactobacilli by an API ZYM system

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In recent years, lactobacilli are widely used in the food industry due to their various organoleptic and nutritional properties. An important factor in the formation of the specific taste and aroma of the fermented food produced with their participation is their enzyme potential. Thereby this study aimed to investigate the enzyme profiles of 25 lactobacilli, isolated from traditional Bulgarian artisanal dairy products. The enzyme activity of the tested strains was determined by using a rapid API ZYM system. Most of the strains showed high leucine and valine arylamidase, β-galactosidase and α-glucosidase activity. Only a few strains had N-acetyl-β-glucosaminidase, α-galactosidase, β-glucosidase and Naphtol-AS-BI-phosphohydrolase activity. Alkaline phosphatase, trypsin, α-chymotrypsin, α-mannosidase, lipase (except Lactobacillus lactis OC2), β-glucuronidase (except Ligilactobacillus salicinus KC2) and α-fucosidase (except Lactiplantibacillus plantarum BS41 and L. lactis OC2) activity were not observed. Obtained results give us only initial information for the metabolic activity of the tested lactobacilli. Therefore, additional analyses are needed and are still in progress.

Acknowledgements: The work is a part of National Program "EUROPEAN SCIENTIFIC NETWORKS" of Ministry of Science and Education of Bulgaria, project "Drug molecule" D01-278/05.10.2020.

Keywords: lactobacilli, enzyme profile, β-galactosidase

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Quartz Crystal Microbalance (QCM): a biosensor to study the microbial biofilm formation in real-time

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Bacterial adhesion is a critical phase in biofilm formation, which needs to be further understood to develop new strategies to promote or prevent biofilm formation.
Bacterial adhesion to a surface is a complex process regulated by quorum sensing, reversible and irreversible adhesion to the surface, extracellular matrix (EPS) and maturation and dispersion of biofilm.
In nature, microorganisms aggregate and associate closely with a surface, forming a biofilm that develops greater resistance to external environmental factors. These aggregations determine problems and loss of efficiency in equipment in the food industry. An alternative technique to study biofilm formation is the Quartz Crystal Microbalance (QCM).
The QCM is a non-destructive flow method that uses an oscillating piezoelectric quartz crystal sensor to measure changes in frequency (Δf) in real-time. The changes in frequency can be related to changes in mass (order to nanograms) through the Sauerbrey equation providing results on the different steps of biofilm formation.
The QCM has been successfully used in this study to evaluate the microbial growth and the phases of the real-time biofilm formation, considering the variation of the resonance frequency (Δf) until 22-24 hours. The microorganisms studied have been Escherichia coli, Staphylococcus spp., and Pseudomonas spp.

Keywords: microbial biofilm, biosensor QCM, surfaces, Escherichia coli, Pseudomonas spp. Staphylococcus spp

[PP-081] DOI: 10.2478/ebtj-2023-0004

Nutrition Value of White Wheat Bread Supplemented with Soybean and Lentil Germinated Flour

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Supplementations of soybean (5, 10, 15, 20%) and lentil (2.5, 5, 7.5, 10%) flours in a germinated form to wheat flours were carried out to test their effects on nutritional value of bread. Also the effect of an optimum mix of 5.60% SGF and 3.62% LGF on bread quality were analyzed. According to our data the protein and fat significant ($p<0.05$) increased with the increase level of germinated flour addition in wheat flour. The carbohydrates content decreased with the increased level of germinated flour addition in wheat flour whereas the moisture content of the bread presented various fluctuations. The energetic value of breads slightly increased when germinated flours were added in bread recipe. The minerals elements analyzed (Ca, Cu, Fe, K, Mg, Mn, Na, Zn) increased with the increase level of germinated flour addition in wheat flour. From the amino acids point of view, high levels were obtained for the essential amino acids histidine and valine and for the non-essential amino acid glutamic acid for all bread samples with germinated flours addition in bread recipe.

Acknowledgments
This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS—UEFISCDI, project number PN-III-P1-1.1-TE-2019-0892, within PNCDI III.

Keywords: germinated soybean flour, germinated lupine flour, nutrition bread quality, health benefits

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A nano-engineered universal antibiotic degradation strategy cum whole-cell biosensor-based degradation monitoring system

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In an effort to diminute the spread of COVID-19 and related bacterial infections, there has been an unprecedented jump in the use of antimicrobials and antibiotics. Macrolides were the most utilized of all antibiotics. Irrational use of macrolides increased their accumulation in water bodies through sewage, and other anthropogenic activities. As a result, continuous exposure of macrolides in aquatic ecosystem has supposedly led to another major threat of Anti-microbial Resistance (AMR) among bacterial species. Here, we devised a nano-biotechnology based affordable, user-friendly, rapid, and robust strategy for detection, mitigation, and degradation of erythromycin, one of the macrolides, present in water bodies. We synthesized iron oxide loaded single-walled carbon nanotubes (MSCN), by simple co-precipitation method, for the purpose of adsorption whereas, bioengineered transcriptionally regulated whole-cell biosensors for detection of erythromycin. Though, MSCN showed good adsorption efficiency, whole-cell biosensor also demonstrated high sensitivity and specificity for erythromycin. To degrade erythromycin after mitigation, heterogeneous Fenton-like reaction was triggered with H_2O_2 as initiator and monitored with biosensor. Other classes of antibiotics were also degraded using this strategy to signify its universality. Hence, this strategy manifests an explicit consideration for keeping check on environmental risk assessment and resistance development for antibiotics.

Keywords: Erythromycin, Single-walled carbon nanotubes, Whole-cell biosensor, Heterogeneous Fenton-like reaction

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Silver Nanoparticles and Vitamin D3 as composition for topic application

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Vitamin D3 or cholecalciferol production occurs in the skin by ultraviolet radiation starting from 7-dehydrocholesterol, located in the keratinocyte of the basal and spinous layer of epidermis. The activation requires hydroxylation process in the liver and kidney to form calcitriol. Recently, it has been reported that vitamin D3 can be activated directly in keratinocyte. We have previously demonstrated that vitamin D3 is able to increase the wound healing respect to the control sample, without however leading to a complete repair. Thus, the need was felt to formulate a preparation that could contain an agent facilitating complete repair. Since silver nanoparticles also showed a partial action in wound repair, it was thought to combine vitamin D with silver nanoparticles. The results showed that this association was capable of inducing a complete wound healing. Moreover, a treatment of vitamin D3 + silver nanoparticles yielded a small percentage of keratinocytes vimentin-positive, suggesting the possibility that the treatment was responsible for epithelial to mesenchymal transition of the cells, facilitating wound healing repair. Moreover the composition increased the level of ceramide, a known molecule with differentiating action. Based on these results, the product was patented by the

Steve-Jones Company. (patent n ° 1020200000009388)

Keywords: cholecalciferol, keratinocytes, silver nanoparticles, wound healing

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Novel Solid Phase Microextraction (SPME) protocol development for non-toxic precise in vitro analysis for drug development and metabolomic analysis

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SPME is a promising technique for early drug development. It has been successfully used for various in vivo and in vitro applications. Moreover, it can be used for more accurate in vitro-in vivo extrapolation. In the course of this study a non-toxic, simple and high-thruput SPME protocol was developed. Fibers covered with 2 mm HLB-based coating were prepared. System was developed using a standard 96-well culture plate. B16F10 and LL2 cell lines were used in the study. After culturing cells for 24, 48, 72 and 96 hours extractions were performed directly from 96-well plate. After the last extraction MTT or SRB assay was performed. The analysis was repeated for combretastatin A4 (CA4) treated cells. Previously developed system for extraction under biosafety cabinet influenced cell growth thus intra-incubator system was developed. The newly developed system did not affect the cells compared to no extraction culturing protocol. Furthermore, analysis of IC-50 of CA4 culture was not influenced by the SPME fibers, proving that developed platform is non-invasive for cells and can be used with standard cell culture assays during drug development process. The project is financed by the National Center for Research and Development (NCBR) under the program MicroIVIVE, project number POLTUR4/MicroIVIVE/5/2021.

Keywords: Early drug development, In vitro-in vivo extrapolation, Solid phase microextraction (SPME)

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Immunohistochemical expression of matrix metalloproteinase-3 and BPV-E2 in bovine cutaneous warts induce by bovine papillomavirus

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Bovine cutaneous fibropapillomas are lesions associated with bovine papillomaviruses. Tumour invasion is associated with increased activity of matrix metalloproteinases (MMPs) which are endopeptidases capable of degrading extracellular matrix (ECM) components. The aim of this study was to immunohistochemically evaluate expression of MMP-3 and BPV-E2 in bovine cutaneous fibropapillomas. Eight bovine fibropapillomas BPV-2 positive and two skin samples were analyzed. The immunostaining was performed by avidin-biotin-peroxidase complex technique. Primary antibodies (anti-MMP-3, Santa-Cruz: 1B4:sc-21732; anti-BPV E2, Santa-Cruz (1E4): sc-57644) were applied overnight. The immunoreactivity for each sample was scored from negative to strong, as follows: n.a., not assessable; negative, weak, moderate and strong positivity. In 5/8 (62,5%) fibropapilloma samples, the majority of keratinocytes and fibroblast cells showed a moderate granular cytoplasmic immunostaining for MMP-3. In skin samples, a similar pattern was described in the cytoplasm of basal cell layer. BPV-E2 protein was expressed perinuclear in the upper layers of fibropapillomas. An impaired expression of MMP-3 in the presence of viral oncoprotein BPV-E2 may play a role in the pathogenesis of the BPV induced tumours; implications of these MMPs in bovine cutaneous fibropapilloma needs to be further investigated. Funding: grant of Ministery of Research, Innovation and Digitalization, CNCS/CCCDI_UEFISCDI, project 50/2020 - PNDI III.

Keywords: MMP-3, fibropapilloma, bovine

[PP-086] DOI: 10.2478/ebtj-2023-0004

GC-MS profiling of volatile metabolites produced by Klebsiella pneumoniae.

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Currently used methods for diagnosing of ventilation-associated pneumonia (VAP) are complex, time consuming and require invasive procedures. Empirical antibacterial therapy in VAP applies broad spectrum antibiotics, that promotes antimicrobial resistance. Hence, the novel and fast methods based on alternative markers are needed for VAP detection and differentiation of causative pathogens. Pathogenic bacteria produce a broad range of volatile organic compounds (VOCs), some of which may potentially serve as biomarkers for microorganism identification. Additionally, monitoring of dynamically changing VOC concentration profiles may indicate emerging pneumonia and allow timely implementation of appropriate antimicrobial treatment.

This study aims at characterization of VOCs release or uptake by carbapenems-resistant and sensitive strains of Klebsiella pneumoniae (KPN). Headspace samples from both cultures types were preconcentrated on multibed sorption tubes and analyzed by GC-MS. Sampling was done under strictly controlled conditions at several time points to follow the dynamic changes in temporal VOC concentration profiles. Altogether 47 VOCs were released by sensitive and 33 by resistant strain, amongst which 2-pentanone, 2-heptanone and 2-nonanone were significantly higher for carbapenem-resistant KPN. This study was financed from National Science Centre project no. 2017/26/D/NZ6/00136.

Keywords: volatile metabolites, bacteria markers, Gas Chromatography – Mass Spectrometry (GC-MS), Klebsiella pneumoniae, Ventilation-Associated Pneumonia (VAP), headspace analysis

[PP-087] DOI: 10.2478/ebtj-2023-0004

Gluten-free appetizer biscuits enriched with Moringa Oleifera powder: Composition, functional and sensorial characteristics

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The effect of replacements of gluten-free (GF) flours with different amounts of moringa powder on the composition, functional and sensorial characteristics of gluten-free appetizer (GFA) biscuits was investigated. The study was conducted on two types of GFA biscuits: oatmeal and rice flour biscuits which constituted the control samples and 8 variants of samples supplemented with 5%, 10%, 15%, and 20% of moringa powder. Due to the addition of Moringa powder, the samples have a significantly higher protein and fiber content than the control samples. By replacing oatmeal and rice flour with 5 to 20% Moringa powder, an upward trend in water absorption capacity (WAC) was observed. The highest value of WAC was recorded for rice flour with a 20% moringa sample. Similarly, the content of moringa powder contributes to the increase of the oil absorption capacity (OAC). Furthermore, rice flour mixed with Moringa 20% has the highest value in this case. Following the research, it was found that panelists accept biscuits with a content of 5% Moringa from a sensory point of view. The results obtained in this study demonstrate that moringa powder can be used to obtain gluten-free appetizer biscuits with a high content of fibers and proteins.

Keywords: Moringa Oleifera, gluten-free, biscuits, functional properties, sensorial characteristics

[PP-088] DOI: 10.2478/ebtj-2023-0004

An innovative vegetable bar coated in alginate-based film enriched with bioactive ingredients

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Adding bioactive extracts or probiotic microorganisms to improve food products' intrinsic value and shelf-life is current practice. This research aimed to obtain a vegetable bar coated with an edible functional alginate-based film enriched with bioactive ingredients and to evaluate the product's sensory, physicochemical, and microbiological properties throughout the shelf life. In this research, some vegetable bars were coated with edible functional alginate-based film enriched with bioactive ingredients and others with collagen film (control samples). The results highlighted that the color changes and pH were insignificant ($p>0.05$) for both samples coated with alginate-based and collagen films. Regarding the spreadability of the products, expressed as "flow threshold", significant statistical differences were obtained ($p<0.05$) due to different thicknesses and permeability of water vapors recorded between the analyzed samples. Compared to the microbiological standards for such vegetable products, it was found that the samples analyzed in this study are microbiologically compliant; they have a reasonably long shelf life, even after 15 days of refrigeration preservation, the bacterial microbiota being below 5×10^4 cfu/ml. In addition, the absence of coliforms is found throughout the analysis. Moreover, the sensory characteristics recorded higher values in the samples coated with alginate-based films than those coated with collagen films.

Keywords: vegetable bar, alginate-based film, bioactive ingredients, shelf life

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The impact of Moringa Oleifera powder addition on total phenolic content and antioxidant activity of gluten-free appetizer biscuits

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Moringa oleifera is a plant rich in specific macro and micronutrients with great importance in human nutrition. It has numerous applications in the food and medical fields due to its antioxidant, antimicrobial, anti-inflammatory, and anti-diabetic potential. The present research was conducted on two types of gluten-free appetizer (GFA) biscuits obtained from rice flour and oat flour, respectively, which were supplemented with different amounts of moringa that varied between 5% and 20%. Therefore, it was evaluated the influence of moringa powder on

the total phenolic compounds (as gallic acid equivalent) and antioxidant capacity (as a percentage of free radical DPPH) of GFA biscuits in comparison with control samples (gluten-free oat biscuits and gluten-free rice biscuits). The experimental data demonstrated the significant influence of moringa powder on the antioxidant capacity and the total content of polyphenols. The highest values were recorded in the case of rice-based biscuits, and significantly higher values were recorded in the case of samples based on rice with the addition of moringa in the proportion of 20%. In conclusion, it can be mentioned that with the increase in moringa content, the values of phenols and antioxidant activity also increase.

Keywords: Moringa Oleifera, gluten-free, DPPH, total phenolic content

[PP-090] DOI: 10.2478/ebtj-2023-0004

Ethanol production from sweet sorghum stem juice under very high gravity fermentation: effect of initial sugar, nitrogen, yeast cell concentrations and temperature

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Very high gravity (VHG) fermentation is a process improvement aimed to increasing both the rate of fermentation and ethanol concentration. However, VHG fermentation can have negative effects on cell growth and ethanol fermentation due to high osmotic pressure. This research aimed to enhance ethanol fermentation efficiency from sweet sorghum stem juice by Saccharomyces cerevisiae NP01 under VHG fermentation. Three main parameters i.e. initial sugar concentration (250-310 g/L), urea concentration (1.6-4.8 g/L) and cell concentration (5.0×10^7-2.0×10^8 cells/mL) were optimized using response surface methodology (RSM) based on Box-Behnken design. The fermentation was carried out at 30°C in a 500-mL air-locked Erlenmeyer flask. The results showed that the optimal initial sugar, urea and cell concentrations for high ethanol production were 267 g/L, 3.24 g/L and 1.32×10^8 cells/mL, respectively. Under these conditions, the highest ethanol concentration (P_E) ethanol productivity (Q_p) and sugar consumption (%SC) were obtained at 121.15 g/L, 2.52 g/L·h and 89.60%, respectively. When the fermentation was controlled at 25°C under these conditions, the P_E and Q_P values decreased to 116.25 g/L and 1.94 g/L·h, respectively. More severe effect on ethanol production under the VHG fermentation was observed at higher temperature of 35°C (P_E, 100.49 g/L and Q_P, 2.09 g/L·h).

Keywords: Very high gravity, ethanol fermentation, Saccharomyces cerevisiae

[PP-091] DOI: 10.2478/ebtj-2023-0004

Oleogels: an alternative to solid fats when making buns from yeasted dough

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The present work aims to replace margarine with a vegetable fat (oleogel) that has similar properties to solid fats but possesses a healthier fatty acid profile. The oleogel was obtained in proportions of 3-11% using Carnauba Wax and Beeswax to sunflower oil and olive oil. For this, dough samples for buns were prepared from a mixture of flour, yeast, salt, water and different proportions of oleogels. The effect of oleogels on the physical properties of the buns was evaluated by analyzing the peroxide index, dough characteristics, texture and rheology. The results obtained when determining the oxidative stability of oleogels show a higher value of the peroxide index for olive oil, which is less stable when storing the oleogel. Dynamic viscoelastic properties of dough samples by replacing margarine with oleogel were investigated using HAAKE RheoWin Mars. The results of the determination of the G'G″ modules demonstrate the increase of the elasticity module with the increase of the oleogel addition. The dough and baked buns were texturally analyzed using the TVT 6700 texturometer. No significant differences in texture were observed between margarine buns and buns made with oleogels. The sensory determination was made using experienced tasters who used the scoring scale.

Keywords: bakery products, solid fats, oleogelators, dough rheology, textural characteristics, sensory analysis

[PP-092] DOI: 10.2478/ebtj-2023-0004

Beyond the ordinary: Zymomonas mobilis super resistance against antimicrobial peptides

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Antimicrobial peptides (AMPs) have been proposed as a promising class of novel antimicrobials and a future alternative to the traditional antibiotics, largely because bacteria are less capable to develop resistance to these compounds. We examined the AMP resistance of an ethanologenic alpha-proteobacterium Zymomonas mobilis, which is known itself as a bacterium with a strong antibacterial and antifungal activty. Comparative evaluation of the antimicrobial activity of 12 different AMPs against a range of Gram-positive and Gram-negative bacteria

surprisingly shows that Z. mobilis possesses hyper-resistance against all those peptides. While the examined peptides exhibited somewhat similar activity against E. coli and all ESCAPE pathogens (E. faecium, S. aureus, K. pneumoniae, P. acne and P. aeruginosa) with characteristic minimal inhibitory concentration (MIC) within the range of 2-20 ug/ml, Z. mobilis demonstrated a significantly higher resistance. Normal growth was retained in the presence of peptides over concentration range from 50 ug/ml to 250 ug/ml. At present, the mechanisms of Z. mobilis super-resistance are not clear, but we speculate that they might be related to some novel defence system, based either on (i) specific properties of the cytoplasmic membrane, (ii) the uncoupled energy metabolism of this bacterium, or (iii) some highly active extracellular proteases.

Keywords: Antimicrobial peptides, resistance, Zymomonas mobilis

[PP-093] DOI: 10.2478/ebtj-2023-0004

An Eco-friendly Approach for the Synthesis of Silver Nanoparticles Using Green Microalga Desmodesmus protuberans

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Over the last decade, novel synthesis approaches for nanoparticles increase their popularity and present interesting area such as energy, medicine, pharmaceutical industries, electronics and space industries in nanoscience and technology. Among different methods to synthesize silver nanoparticles, the biological method has been the most intensive investigated. Microalgae can also be used in Bio-NP synthesis which is relatively unexplored, but it is more biocompatible an alternative method than the other biological methods. The aim of this study was the evaluation of the ability of selected microalgae to biosynthesize silver nanoparticles. The green synthesis of AgNPs was confirmed by the color change from colorless to yellowish brown. The biosynthesis of AgNPs was further confirmed by UV-Vis absorption spectroscopy, scanning electron microscopic (SEM) and energy dispersive X-ray analysis (EDX). It was observed that the algal cells synthesized under SEM analysis are intact and the nanoparticles accumulate in the compact inner region of the cells without forming aggregates. The characteristic absorption peak at 450 nm in UV–vis spectrum confirmed the formation of silver nanoparticles. Energy dispersive X-ray spectroscopy revealed strong signals in the silver region and confirmed of the AgNPs.

Keywords: silver nanoparticles, microalgae, Green synthesis, Desmodesmus protuberans

[PP-094] DOI: 10.2478/ebtj-2023-0004

Study on the potentiation of the cytostatic action of Cisplatin by association with near infrared radiation (NIR)

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Cancer, also known as malignant neoplasm or malignant tumor, represents a group of diseases that involve the uncontrolled and continuous multiplication of abnormal cells, originating from any tissue of the human or animal body. From a clinical point of view, the differences between tumors are determined by the mode of onset, growth rate, diagnosis, detectability, potential for invasion, metastasis, response to treatment and prognosis. The study developed by us was carried out on outbred Wistar rats, carriers of subcutaneous Walker 256 carcinosarcoma, and is based on the positive photodynamic potential of some cytostatics, including cisplatin. The obtained results demonstrated that by potentiating cisplatin with the infrared radiation emitted by a Bioptron type device, with a wavelength (λ) of 780–1100 nm, we managed to increase its therapeutic index by: i) decreasing the total dose of cisplatin, simultaneously with the decrease of side effects; ii) preventing the installation of chemo-resistance by increasing the therapeutic index and limiting the number of treatments; iii) the possibility of destroying both tumor cells in division and those in G phase using photodynamic "photokilling" processes.

Keywords: Walker 256 carcinosarcoma, Cisplatin, near infrared radiation, therapeutic index

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The possible role of peroxisomes in transition into the state of anhydrobiosis and following reactivation

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Peroxisomes are dynamic organelles. They are involved in various metabolic pathways that predominantly depend upon cellular and environmental conditions. Yeasts are convenient model to study peroxisomes because cell transfer from a glucose containing medium to a medium containing a peroxisome

proliferator (oleate, methanol) induces synthesis of peroxisomal enzymes and the growth and division of peroxisomes. Extensive knowledge has been gained about the role of the plasma membrane and its proteins in the transition of yeast cells into the anhydrobiosis state. Nothing is known about other organelles, like peroxisomes, during stress response. The aim of this study was to obtain first information concerning the role of peroxisomes in the transition of yeasts into the state of anhydrobiosis and following reactivation. We used Saccharomyces cerevisiae and Ogataea polymorpha strains with affected peroxisome division (pex11Δ), inheritance (inp1Δ), biogenesis (pex3Δ) and peroxisomal matrix protein import (pex6Δ). We concluded that yeasts with intact and functional peroxisomes after their induction are able to withstand better dehydration and subsequent rehydration stress. Overall, our results showed for the first time that if yeast cells were incubated in a medium with a peroxisome inducer before transfer into the state of anhydrobiosis, it gave advantage of better withstanding dehydration-rehydration stress.

Keywords: Saccharomyces cerevisiae , Ogataea polymorpha , peroxisomes, anhydrobiosis

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Escherichia coli O157:H7 death rate following carvacrol exposure: cellular membrane disruption and cellular DNA leakage

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Carvacrol, a plant-derived antimicrobial, has a strong antibacterial activity. For validation the effect of carvacrol solutions (0.20 mg/ml, 0.40 mg/ml) on E. coli O157:H7 were studied concomitantly via leakage degree of cytoplasmic constituents, namely 260nm absorbing material (AM) expressed as cellular DNA, the cells' microtopography testing, and survivability of E. coli O157:H7. The damage to the bacterial cytoplasmic membrane, measured by the leakage of cellular DNA material, indicated that the cytoplasmic membranes were damaged and unable to retain the cytoplasmic constituents. After 0.2 minutes of exposure time, 0.13 units of DNA material was leaked for 0.2 mg/ml carvacrol concentration, and 0.20 units of DNA material was leaked for 0.4 mg/ml carvacrol concentration. The damage degree of the cytoplasmic membrane and the released quantity of DNA material increased with the length of the carvacrol exposure time. Negative staining and transmission electron microscopy provided further evidence for cytoplasmic membrane damage of the cells exposed to different carvacrol concentrations (0.20 mg/ml, 0.40 mg/ml). Transmission electron microscopy revealed that the carvacrol exposed E. coli O157:H7 cells became wrinkled, collapsed, and loss of cellular constituents. E. coli O157:H7 survivors decreased with increment of the time of exposure to carvacrol solutions and carvacrol concentrations.

Keywords: Carvacrol, Escherichia coli O157:H7, Antimicrobial

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Immunofluorescence evaluation of anti-cROS-1 and anti-PARP-1 antibody expression in vesicant chemical burns

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Objective: to evaluate the cROS and PARP-1 cutaneous expression by immunofluorescence technique after exposure to chemical vesicant agents.

Method

Experimental study was performed with the approval of the Ethics Commission; three study groups of five rats each were used: control group, unexposed; exposed group to vesicant; exposed group to vesicant and treated with a complex formula consisting in anticytokines, PARP inhibitors, growth factors (EGF, VEGF) Results: Control group: the expressions of the anti-cROS-1 and anti-PARP-1 antibodies were positive in rare basal cells. Exposed and untreated study group: anti-cROS-1 antibody reaction was positive in endothelial cells of the hypoderm. Anti-PARP-1 antibody is activated in the reepithelialization area, as a response to DNA repair. Exposed and treated study group: the hypodermic vessels stopped being positive for the anti-c-ROS-1 antibody (their neoformation-mediated role has been completed); the expression of anti PARP-1 was inhibited (the nuclear apparatus remained intact under the protection of the treatment). Conclusions: the skin toxicity of vesicants is highlighted by severe cell aggression. At the molecular level, the activation of anti-cROS-1 and anti-PARP-1 antibodies is found. The protective effects of the treatment is correlated with the inhibition of anti PARP-1 expression and a weaker expression of anti c-ROS-1 antibodies.

Keywords: vesicants, anti c-ROS-1, anti-PARP-1, anticytokines, growth factors

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Sugarcane bagasse and straw as feedstocks in the production of xylitol: Cellular adaptation as a strategy to improve this bioprocess

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One of the key steps of the biotechnological production of xylitol – a versatile molecule with applications in different industrial segments - is the deconstruction of the plant cell wall. Lignocellulosic inhibitors formed/released at this step, such as furans and phenolic compounds, inhibit xylose-to-xylitol bioconversion. This work aimed to evaluate the adaptation of Candida tropicalis to sugarcane bagasse and straw mixture (1:1) hemicellulosic hydrolysate (SBSHH) to overcome the toxicity of lignocellulosic inhibitors and improve the hydrolysate fermentability. Fermentations were performed in concentrated/detoxified SBSHH using yeast adapted to SBSHH obtained by dilute acid hydrolysis with different chemical characteristics (original, concentrated, and concentrated/detoxified). Yeast adaptation to concentrated/detoxified SBSHH increased xylitol yield and xylose-to-xylitol conversion efficiency by 13.3 %, xylitol volumetric productivity by 7.1 %, and xylitol specific production rate by 9.7 %. An improvement in 5-HMF degradation compared to non-adapted cells was also observed. Cells adapted to all three hydrolysates exhibited a decrease in total phenolic compounds reduction throughout fermentations, indicating possible physiological changes due to cellular adaptation. It is concluded that the adaptation of C. tropicalis to concentrated/detoxified SBSHH was the most effective strategy to improve xylitol production.

Keywords: Sugarcane bagasse and straw hemicellulosic hydrolysate, Lignocellulosic inhibitors, Cellular adaptation, Candida tropicalis, Xylitol

[PP-099] DOI: 10.2478/ebtj-2023-0004

Employment of thermophilic bacterium Schlegelella thermodepolymerans in biodegradation of various biopolyesters

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Mass glorification of biodegradable materials leads to an increase in new plastics appearance, which are expected to undergo rapid degradation processes under natural conditions. With popularity, biodegradable plastics are employed in the consumer products packaging, production of sacks and bags or food serving. Nevertheless, the infrastructure for waste bioplastics treatment has not yet been developed. Since the biodegradable waste is metabolized into methane in the anaerobic conditions, it deserves a separate way of disposal. One of the most promising methods for bioplastic recovery is common composting. The biodegradation is performed by a wide range of microbial strains capable of producing extracellular hydrolases. Amongst the most promising bacterial strains degrading biopolymers is Schlegelella thermodepolymerans. This thermophilic strain was found to be capable of biodegradation of even degradation-resistant biopolymers containing thioester bonds. Within this study, we have investigated the degradation activity of S. thermodepolymerans on several conventional biopolyesters, such as polyhydroxybutyrate, polylactic acid, polycaprolactone etc. Further, the degradation kinetics and morphological changes of the degraded specimens were described. Since the degradation tests are provided in thermophilic conditions, the prevailing mechanism of degradation is evaluated. Based on the experimental results, the suitability of S. thermodepolymerans employment in the bioplastic waste treatment is discussed.

Keywords: biopolyesters, bioplastics, waste treatment, Schlegelella thermodepolymerans, bioinoculant

[PP-100] DOI: 10.2478/ebtj-2023-0004

Agrobacterium-mediated CRISPR/Cas9 genome editing in elite line of Chinese cabbage (Brassica rapa) for TuMV resistance

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Trait advancement by genome engineering and editing approaches particularly CRISPR technology is becoming state of art in numerous breeding programs. Agrobacterium-mediated stable expression and virus mediated transient expression of CRISPR/Cas9 system are evolving in the genomics assisted breeding of horticultural crops. In this study, we attempted to develop varieties of TuMV (turnip mosaic virus) resistant Chinese cabbage using CRISPR/Cas9 based gene editing. For the editing, guide RNA of eIF(iso)4E gene which is a well-known TuMV-resistant gene is employed. The plasmid vector was constructed with Cas9 gene along with gRNA and Agrobacterium-mediated transformation was performed in elite line of Chinese cabbage. To confirm the existence of eIF(iso)4E gene, genomic DNA was isolated from leaves of transgenic (T_0) plants and confirmed using PCR. Further validation of transformants by deep sequencing analysis was conducted on the transgenic plants. The results of the current study can be extended for the development a potential CRISPR/Cas9 system in horticultural crops to develop new varieties using genome editing techniques.

Keywords: Chinese cabbage, gene editing, TuMV, resistance

[PP-101] DOI: 10.2478/ebtj-2023-0004

Development of UPR gene mutated Chinese cabbage related to cell immortalization using CRISPR/Cas9 system

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Chinese cabbage is a Brassica crop such as Arabidopsis, the Arabidopsis expression system can directly be used, and the genome and transcript sequences have been reported, so it can be used for gene editing. There have been many studies on the production of biopharmaceuticals using plants, but there is a limit to the low productivity and improvement of the protein production yield in plant cells. Therefore, in this study, we tried to obtain a Chinese cabbage mutant by inducing a mutation in the UPR gene, which is a fundamental adaptive cellular response to various internal and environmental stresses in plants using gene editing technology. Chinese cabbage transformants were obtained using Agrobacterium-transformation and CRISPR/Cas9 gene editing system. The presence/absence of gene insertion was performed through PCR analysis, genetically mutated-plant were selected through deep-sequence analysis, and the homozygous was obtained through self-fertilization. The obtained E_1 seeds were sown in media treated with different concentrations of tunicamycin, an n-glycosylation inhibitor, and stress tolerance was compared with wild-type plants 7 days later. As a result of fresh weight and ER stress analysis, there was a significant difference with wild-type, which is likely to be significant for stress tolerance and proliferation of plant cells.

Keywords: Chinese cabbage, CRISPR/Cas9, Agraobacterium-transformation, UPR gene

[PP-102] DOI: 10.2478/ebtj-2023-0004

Antibacterial activity of extracts from the fungus species Lepista personata against Escherichia coli NBIMCC K12 and Bacillus subtilis NBIMCC 3562

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Nowadays, fungi are famous for the production of various bioactive substances which have beneficial properties for human health, like antibacterial, antifungal, antioxidant, antitumor, cytostatic, and etc. The aim of the present study is to determine the antibacterial activity of different (hot and cold ethanol/dichloromethane/hexane) extracts from the fungus species Lepista personata against Escherichia coli NBIMCC K12 and Bacillus subtilis NBIMCC 3562. The antimicrobial assays were performed in triplicate by using the classical disc diffusion method. Extracts were previously dissolved in 200 mM dimethyl sulfoxide, which was used as a control sample. Results showed that E. coli NBIMCC K12 is susceptible to dimethyl sulfoxide whereas the growth of B. subtilis NBIMCC 3562 was not affected. All fungus extracts demonstrated no inhibitory activity against B. subtilis NBIMCC 3562. At the same time, only the dichloromethane extract obtained after hot extraction inhibited the growth of E. coli NBIMCC K12.

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Keywords: inhibitory activity, bioactive substances, antibacterial

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Pectin extraction from sugar beet flakes: study of the pretreatment of plant material and influence of the extraction agent

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The aim of this research was to find the best choice of pretreatment applied to sugar beet flakes before pectin extraction, and to investigate the influence of the extraction agent on the yield of the process and the purity of the extracted pectin. Two different pretreatment methods were applied: a drying process, conducted at 60 °C in an oven with air circulation until constant weight, and a lyophilisation treatment, that was performed at -50 °C and pressure of 4.2 Pa for 12 h. After pretreatment, from the resulting material pectin was extracted using two different acids, citric acid and hydrochloric acid, at a pH of 1.5, solid-to-liquid ratio of 1:15 w/w, temperature of 90 °C and 120 min extraction time. Overall, better results were achieved when citric acid was used as extraction agent, and thereby in the second part of the study citric acid was selected as extraction agent and the extraction was performed using the powder from both pretreatment methods, on three separate samples for each pretreatment according to particle size intervals.

Acknowledgement
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Keywords: sugar beet flakes, pectin, extraction, pretreatment

[PP-104] DOI: 10.2478/ebtj-2023-0004

TiO2 nanoparticles affect morphology and cytokine secretion in human macrophages

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Titanium (Ti) belongs to the most common materials used in implantology. However, due to friction and wear of the implants, Ti nanoparticles (NPs) can be released and transported into the bloodstream. In this work, the effects of TiO2NPs on the morphology and functional status of human macrophages (Mφ) were studied. Mφ were isolated from whole blood samples of patients with osteoarthritis undergoing joint replacement surgery. Cell viability was determined by the trypan blue exclusion assay, where after 5 days of cultivation with TiO2NPs, the viability was higher than 88%. However, the addition of TiO2NPs results in the formation of a larger number of cells with an elongated spindle-like morphology. When analyzing F-actin expression, a 37% reduction in expression was observed. Then, M1 and M2 macrophage phenotypes were examined using the surface markers CD86 and CD206, with no changes detected. Further analysis of inflammatory cytokines and chemokines revealed that the secretion was increased by nanoparticle treatment. The results of study show that TiO2NPs cause changes in cell morphology and lead to Mφ activation. These findings could help to understand the potential toxic effects of wear debris on macrophages after implantation of Ti-based endoprostheses.

Keywords: Ti nanoparticles, implantation, osteoarthritis, macrophage, toxicity

[PP-105] DOI: 10.2478/ebtj-2023-0004

Characterization chemical and biochemical of the biosurfactant produced in the biorefinery context

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Biosurfactants are amphipathic molecules, biodegradable, with reduced toxicity. They can be synthesized by fermentation processes from oleaginous compounds and agro-industrial by-products. In this context, the present work aims to realize the characterization of the biosurfactant produced in a culture medium composed low cost substrates. For this, a biochemical analysis of the obtained biosurfactant was performed, by spectrophotometric methods, and FTIR. From these experiments, it was observed that the biosurfactant produced has about 53 % of carbohydrates, 40 % of proteins and 6 % of lipids in its com position, characterizing it as a polymeric molecule. The FTIR spectrum confirms the presence of functional groups such as amides, amines, and carbonyl. The emulsifying property was also studied. It was observed that the biomolecule has a better emulsifying action in organic solvents with non-polar character. Thus, the biosurfactant produced under the applied conditions was classified as a polymeric biosurfactant and has an outstanding emulsifying property in organic solvents. Therefore, it is expected that this biomolecule is a potential substitute for synthetic surfactants and that it can be used in different applications.

Keywords: biosurfactant, agro-industrial, low cost substrates, polymeric characterization

[PP-106] DOI: 10.2478/ebtj-2023-0004

VALORIZATION OF SUGARCANE BAGASSE AS A POTENTIAL SUBSTRATE IN THE PRODUCTION OF BIOEMULSIFIER BY Aureobasidium pullulans LB83

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In the present study, the bioconversion potential of sugarcane bagasse by the yeast Aureobasidium pullulans LB83 was evaluated for the production of a bioemulsifier in solid-state fermentation (SSF). The sugarcane bagasse was submitted to alkaline pre-treatment, in the proportion of 0.12 (g/g) of NaOH/bagasse and 12 min in autoclave for lignin removal. After chemical analyses, fermentations were carried out in Erlenmeyer flasks containing 2 g of pretreated sugarcane bagasse (PSB), used as substrate. The flasks were inoculated with a concentration of 108 cells/mL, a moistening solution and incubated for 12 days at 28°C. Triplicates were collected every 48 h to obtain the cell-free metabolic fluids that were used to determine the emulsification index (IE24%) with hexane and soybean oil. The results obtained showed that, under the established conditions, the pre-treatment showed a lignin removal of 74.7%. The maximum value of emulsification index was observed on the eighth day with an emulsification index of 53.1% and 42.5% for soybean oil and kerosene respectively. These results showed the possibility of producing a value-added product as a bioemulsifier from the pretreated sugarcane bagasse. Likewise, the data obtained suggest a possible application of the biomolecule in the food industry.

Keywords: Lignocellulosic biomass, bioemulsifier, alkaline pretreatment, emulsification index

[PP-107] DOI: 10.2478/ebtj-2023-0004

Partial Characterization of Bacteriocin-like Compounds Isolated From Petroselinum crispum Fermented Juice

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The aim of this work was to find out if Staphylococcus carnosus, has an efficient fermentation process to generate bacteriocins, in parsley (Petroselinum crispum) juice and to partial characterization the biosynthesized bacteriocins. Parsley roots juice, inoculated with 107–108 CFU/mL Staphylococcus carnosus at 37 °C for 24±2 h, was centrifuged. The supernatant free of cells was used for isolation and partial characterization of the bacteriocins. The supernatant peptides were precipitated with 60 – 90% ammonium sulfate, collected by centrifugation, dissolved in phosphate buffer (0.01 M PB, pH 7.0), and dialyzed overnight. Heat treatment and pH sensitivity of dialyzed fractions of bacteriocins (DFB) was performed.

The antimicrobial activity of DFB was determined by agar well diffusion assay, using Listeria monocytogenes, as indicator bacteria. The results revealed that 90% ammonium sulfate precipitation fraction showed higher antimicrobial activity. Heat treatment (30 - 70 °C) has no effect on DFB. Temperature > 90 °C reduced the antimicrobial activity of DFB.

In conclusion, DFB exhibited good thermal stability for temperatures between 30-70 °C. The pH stability was good for 4.00-10.00 pH values, but extreme pH values had an inhibition effect on bacteriocins' stability.

This work was supported by the Romanian UEFISCDI projects PN-III-P2-2.1-PTE-2021- 0508 and PN-III-P2-2.1-PED2021-2001.

Keywords: Staphylococcus carnosus, bacteriocin-like Compounds, pH stability, thermal stability, salt stability, antimicrobial activity

[PP-108] DOI: 10.2478/ebtj-2023-0004

Protective effect of fermented parsley root juice (Petroselinum crispum) on minced pork protein and lipids during refrigeration

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Chemical compounds with antioxidant and antimicrobial activities are used to protect proteins and lipids from meat and meat products. To reduce the risks of these chemicals, researchers are looking for solutions that lead to clean label products. This research aimed to determine the protective effect of fermented parsley juice (Petroselinum crispum) (FPRJ) on proteins and lipids in minced pork during refrigeration. For the study, minced pork was treated with either FPRJ in different concentrations. The samples were individually wrapped in polyethene foil and then refrigerated at 4 °C for 8 days. Levels of carbonyl protein, myoglobin, TBARS, conjugated dienes and trienes were determined on days 0, 2, 4, 6, and 8. The results showed that the FPRJ protect proteins and lipids against oxidative processes in a juice concentration-dependent manner. At concentrations of 3 and 4 g/100 g of minced pork, FPRJ had a protective effect on proteins and lipids higher than sodium nitrite.

In conclusion, fermented parsley roots juice, in the concentration of 3 – 4 g/100 g of minced pork, can be used to maintain the integrity of proteins and lipids in minced pork during refrigeration.

This work was supported by the Romanian UEFISCDI projects PN-III-P2-2.1-PTE-2021- 0508 and PN-III-P2-2.1-PED2021-2001.

Keywords: fermented juice, protein carbonyl, myoglobin, TBARS, conjugated dienes, conjugated trienes

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Environmental stress responses in lactic acid bacteria strains isolated from traditional Romanian fermented products

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Spontaneous fermentation has been applied for thousands of years as a method for food preservation. Lactic acid bacteria (LAB) are an important part of the microbiota of fermented food and beverages from all over the world due to their contribution to preservation of food and also for giving these products desirable textures and aromas. When selecting strains for food fermentation, different aspects, including safety, functional and technolog-

ical characteristics, have to be taken into consideration. The aim of the present study was to assess growth parameters profile in different environmental stress conditions of some LAB strains isolated from traditional Romanian fermented products. In order to achieve the main objective of this study, the growth of 10 newly isolated LAB strains- previously characterized and taxonomically classified into Lactobacillus, Leuconostoc and Enterococcus genera, selected for their biotechnological potential- was evaluated in different stress conditions (low/high initial pH, reduced/increased growth temperature or NaCl concentrations). The results of the present study revealed that the growth requirements of LAB strains are different and complex and helped us to select the best adaptative strains for further studies concerning the production of several metabolites important for food texture or health (exopolysaccharides or biologically active compounds).

Keywords: lactic acid bacteria, food fermentation, environmental stress conditions

[PP-110] DOI: 10.2478/ebtj-2023-0004

A patient with Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome due to 16p11.2 deletion: a case report from Çanakkale

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Mayer-Rokitansky-Küster-Hauser(MRKH) syndrome describes a spectrum of Mullerian duct anomalies characterized by congenital aplasia of the uterus and upper 2/3 of the vagina. Its etiology is unknown. It is usually diagnosed in adolescence because of primary amenorrhea and its prevalence is approximately 1:4000-5000 in newborn females. 16p11.2 deletion syndrome is a well-known deletion syndrome that can present with various clinical phenotypes, including developmental delay, intellectual disability, autism spectrum disorder, and obesity. We aim to underline the genetic etiology of MRKH syndrome and genitourinary abnormalities in patients with 16p11.2 microdeletion. A 26-years-old woman was referred from the gynecology department with primary amenorrhea, agenesis of uterus and proximal vagina. Her intelligence was normal, and BMI(Body Mass Index) was 35.6. GTG band karyotyping showed 46, XX normal. MicroArray (IlluminaiScanSystem 700K) analysis revealed 1 MB heterozygous deletions encompassing the morbid OMIM genes KIF22, PRRT2, TBX6 region (arr[GRCh38] 16p11.2(29304558-30336410)x1). Similar deletions have been linked to MRKH before. TBX6 functions in mesodermal development and is proposed for the etiology of MRKH in the literature. Our report confirms that MRKH can be caused by 16p11.2 deletion; therefore, we recommend genitourinary system evaluation in patients with 16p11.2 deletion and microarray analysis should be considered in primer amenorrhea patients.

Keywords: MRKH, primary amenorrhea, 16p11.2

[PP-111] DOI: 10.2478/ebtj-2023-0004

New opportunities for sustainable development through capitalization of spruce sawdust to obtain bioethanol

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Annually, large quantities of lignocellulosic biomass are generated from the maintenance of forests or from forestry operations (chips, branches, and sawdust); this biomass represents one of the most promising renewable raw materials for the production of second generation bioethanol due to its high energy value and low acquisition costs. This study aimed to use spruce sawdust to obtain bioethanol. A conversion system of this raw material was implemented, and it comprised a pretreatment step to separate cellulose and hemicellulose from lignin, followed by an enzymatic hydrolysis step of holocellulose to release fermentable carbohydrates, and finally a fermentation step of carbohydrates. The structural changes determined by pretreatment were investigated by scanning electron microscopy and infrared spectroscopy, and the liquid fraction was analyzed in terms of phenolic compounds and organic acids composition. The liquid fraction resulting from enzymatic hydrolysis was analyzed to determine the carbohydrate composition. The concentration of bioethanol that was achieved through the distillation of the mash was determined by gas chromatography.

Acknowledgment

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Keywords: spruce sawdust, pretreatment, enzymatic hydrolysis, fermentation, bioethanol

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Effect of different elicitors on metabolic gene expression and centelloside production in Centella asiatica hairy root cultures

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Centella asiatica (L.) Urban is a relevant plant that have pharmacological properties. These benefits are from secondary metabolites called as centellosides. The main issue is C. asiatica produce them in small amounts. The present study aims of overcoming this limitation, we compared the effect of different elicitors on centelloside production and triterpene pathway gene expression in hairy root cultures established by Agrobacterium rhizogenes infection. The elicitors studied were coronatine and methyl jasmonate, added separately or together, and salicylic acid. The content of the four main centellosides (asiaticoside, madecassoside, asiatic acid and madecassic acid) was determined by HPLC/DAD, and the expression level of key biosynthetic genes after elicitation was analyzed by real-time quantitative polymerase chain reaction. The greatest increase in centelloside production, especially madecassoside, was achieved with coronatine, applied alone or with methyl jasmonate (116 and 125 mg/g DW, respectively). This treatment also enhanced the expression of the target genes, particularly at the start of elicitation. By far the most highly expressed were those involved in oxidations, suggesting that the elicitors tested did not specifically act on key early genes in centelloside biosynthetic pathway. These results support those hairy roots as a promising biotechnological platform for enhancing centelloside production.

Keywords: Hairy roots, elicitation, biosynthetic pathway, centellosides, Centella asiatica

[PP-113] DOI: 10.2478/ebtj-2023-0004

Elicitation-induced metabolic changes in Taxus baccata cell cultures: insights into the control of taxane metabolism

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Paclitaxel (PTX) is one of the most effective anticancer drugs in current use, but more research is needed to understand its molecular/cellular control production in Taxus spp. cell cultures. According to this study, this compound was yielded 11-fold more after elicitation with coronatine (COR) than untreated cells, and 18-fold more when combined together with methyl-b-cyclodextrins (b-CDs). A greatly increase in taxane release from the cells was observed after the dual treatment, with 81.6% of the total content in the medium at the end of the experiment. These experimental conditions also significantly enhanced the expression of taxane biosynthesis genes, especially the flux-limiting genes BAPT and DBTNBT. Recently, it was demonstrated that lipid droplets (LDs) are involved in transporting and accumulating taxanes in Taxus spp. cell cultures. Therefore, their structure, number, and capacity for storing taxanes was also examined. In elicited cultures, the number of LDs increased and they mainly accumulated taxanes with a side chain, especially PTX. Hence, PTX accounted for up to 50-70% of the total taxanes found in LDs in the COR + b - CD-treated cultures. The results of this study confirm that LDs are capable of storing and distributing taxanes within and outside of cells.

Keywords: Taxus baccata, cell cultures, paclitaxel, coronatine, lipid droplets, taxane accumulation

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DNA methylation as a key player in taxanes biotechnological production

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Paclitaxel, an antineoplastic agent widely used to treat different types of cancer, is scarcely distributed in nature. Biotechnological platforms, based on in vitro cell cultures of Taxus spp., are eco-friendly alternatives to its production. Nevertheless, the progressive yield reduction during its in vitro maintenance over time limits and compromise their efficient use. To understand how DNA methylation influences taxane production, we compared methylation patterns between a new line and one of approximately 14 years old. The promoters of three genes (GGPPS, TXS, and DBTNBT) involved in taxane biosynthesis were studied. Our results revealed that each promoter has different strategies controlling its expression. While the core promoter of the GGPPS gene is protected from cytosine methylation accumulation avoiding gene silencing, the DBTNBT core promoter of the old line is completely methylated. In addition, the presence of methylation hot-spots in predicted MeJA-related transcription factor binding sites showed that their action would be compromised in the old line. These results evidence not only the existence of specific epigenetic regulatory mechanisms but also that DBTNBT gene is a key in the paclitaxel biosynthesis.

Keywords: DNA methylation, cis-elements, epigenetic regulation, paclitaxel, promotors, taxane biosynthesis

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Antifungal Assay of Silver Nanoparticles Biosynthesized

using Green Microalga Desmodesmus protuberans

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In comparison to bigger particles, nanoparticles are gaining recognition as complex materials with innovative or cutting-edge properties. Silver nanoparticles (AgNPs) are a new kind of material with several applications, such as sensors, catalysts, anticancer agents and antimicrobial agents. AgNPs have exhibited activity against bacteria, fungi and viruses. Use of biological organisms such as bacteria, fungi, yeast, plant extract or plant biomass, and algae extract or biomass could be an alternative to these methods for the synthesis of nanoparticles in an eco-friendly manner, less time consuming, and low cost. The aim of this study was to synthesize silver nanoparticles using the Green Microalga Desmodesmus protuberans and evaluate its antifungal activity. The morphological characteristics were analyzed by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). Antifungal activity was performed by agar well diffusion method against various pathogenic fungi.

Keywords: green synthesis, marine algae, antifungal activity, Desmodesmus protuberans

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Functional profiling of SARS-CoV-2 B-cell repertoires from vaccinated individuals

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The ongoing SARS-CoV-2 pandemic requires development of rapidly and efficiency technologies to discover novel coronavirus-neutralizing antibodies as potential therapeutics. Here we described novel approach for generation of neutralizing antibodies from blood samples of individuals immunized by mRNA or adenovirus-based vaccines. At the first step of the method the total B-cells isolated and compartmentalized as an individual cells in microfluidic droplets of water-in-oil emulsion with subsequent immobilization of total RNA from a single cell on magnetic beads in the same droplet. At this stage the variable domains of immunoglobulins heavy (VH) and light (VL) chains from each cell are physically linked by ePCR. Further, the resulting VH-VL single chains libraries have been screened using yeast display to isolate Abs against SARSCoV2. The useful advantage of this system is the ability to screen resulted natively paired library to any SARS-CoV-2antigens, including new SARS-CoV-2 variant. We utilized two variants of yeast display to select different populations of Abs – competitive binding (in the presence of ACE2) and binding with extremely low antigen concentration. As a result, we selected panel of antibodies which are neutralize the SARS-CoV-2 virus in Vero cell assay with sub nanomolar constant. This work was supported by grant No. 0751520211049.

Keywords: SARS-CoV-2, virus-neutralizing antibodies, microfluidic platform

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Biological treatment of grape pomace by Rhyzopus oryzae – scale up in a tray bioreactor

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Biological treatment of grape pomace by R. oryzae was carried out in laboratory jars under solid-state fermentation conditions and the process was scaled-up in a tray bioreactor. The aim of this work was to investigate the influence of R. oryzae on the recovery of phenolic compounds from grape pomace, chemical composition and activity of lignolytic and hydrolytic enzymes. A 15-day biological treatment with R. oryzae decreased the content of total phenolic compounds, total flavonoids, and total proanthocyanidins by 47 %, 43 % and 62 % in laboratory jars and by 34 %, 21 % and 42 % in a tray bioreactor, while it had a positive effect on the extractability of 10 of the 21 phenolic compounds quantified by the UHPLC method. Their content increased by 1.1- to 2.5-fold. After 15 days of fermentation in jars and tray bioreactor, respectively, a decrease in reducing sugar concentration by 53 % and 69 % and an increase in fat content by 44 % and 30 % were also observed. Of the enzymes studied, the highest activity was measured for xylanase (62.96 U/gdb).

This work was supported by Croatian Science Foundation [grant number IP-2018-01-1227] and European Regional Development Fund [grant number KK.01.1.1.04.0107].

Keywords: solid-state fermentation, grape pomace, R. oryzae, phenolic compounds, scale up

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Isolation, production and characterization of Cellulolytic Aspergillus sp. that isolated from kombucha tea simbiosis

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Present study was initiated to isolate, produce and characterization of cellulase from kombucha tea simbiosis. Cellulase

producing strains were isolated from kombucha tea simbiosis. Aspergillus sp. AC-5 was the most active cellulase producers. The Aspergillus sp. AC-5 was presented optimum growth at mesophilic temperature. Maximum cellulase production was observed at pH 9 and 30°C. When the enzyme incubated at 20-80°C for 30 second, enzyme had %87 remanied activity at 40°C and % 100 at 30°C. The enzyme was active up to pH 11.0. After incubation for 15 second with enzyme inhibitors and metal ions, the cellulase activity was inhibited by KCl(%60), ure (%72), EDTA (%7), Triton X-100 (%4), ZnCl2 (%2), β-Merkaptoethanol (%32), MgCl2 (%1) and stimulated by CaCl2 (%126), Na2SO3 (%101), PMSF (%101), SDS (%120). The results showed that cellulolytic Aspergillus sp. AC-5 is useful for mesophilic and alkaliphilic case in industrial applications.

Keywords: Cellulase, Aspergillus sp., Kombucha, Enzyme

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Biodegradation of azimino-benzene with glutathione peroxidase as biocatalyst and farther degradation of azimino-benzene with g-C3N4-BaBiDyO4 as catalyst

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The inchoative biodegradation results of azimino-benzene were obtained with glutathione peroxidase as biocatalyst. The results showed that the maximal removal percentage of azimino-benzene was 64.1% after 32 min of reaction time and the upmost experimental parameters were as following: hydrogen peroxide 102.8 μmol/L, enzyme 72.4 U/mL, pH value 4.9 and azimino-benzene concentration 41.2 mg/L. Lactuca sativa and Artemia salina were utilized for estimating the poisonousness of the products. The glutathione peroxidase was very efficient for removing the azimino-benzene, and the products which were achieved after enzymatic removal showed high poisonousness. The lethal concentration LC50 which was acquired for Artemia salina was 46.5% and the inhibition concentration IC50 which was obtained for Lactuca sativa was 27.9%. Above results showed the importance of toxicologic reckon after enzymatic treatment. Farther degradation of azimino-benzene was achieved. The mulriple catalyst g-C3N4-BaBiDyO4 was prepared first. Entire photocatalytic degradation of azimino-benzene with g-C3N4-BaBiDyO4 as catalyst was achieved after visible light irradiation of 48 min. The photocatalytic degradation of azimino-benzene followed the first-order reaction kinetics. The degradation pathway of azimino-benzene was obtained. Acknowledgments: This work was supported by the Team Foundation for Middle-aged and Young Scientist of Science and Technology Bureau of Jilin Province of China (Grant No. 20200301033RQ).

Keywords: Biodegradation, azimino-benzene, biocatalyst, g-C3N4-BaBiDyO4, catalyst

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Production of co-cultivation biomass of carotenogenic yeast and microalgae or cyanobacteria cultivated on media with waste lipid substrates

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The co-cultivation of carotenogenic yeasts and microalgae or cyanobacteria is the effective use of non-competing microorganisms that are capable of exchanging gases and substances that are crucial for survival of both microorganisms. Cultivations were performed on mineral media with waste substrates as a carbon source, such as waste frying oil or waste coffee oil. The carbon source was used to provide nutrition for the yeasts, which during their active aerobic metabolism utilized the given substrate for the intensive production of biomass and many valuable substances (lipids, carotenoids, ergosterol, ubiquinone). The waste product of primary yeast metabolism was carbon dioxide, which was processed by autotrophic algae or cyanobacteria to produce biomass and other valuable metabolites (chlorophylls). At the same time, oxygen, obtained in photosynthesis, was intensively consumed by the yeasts for its propagation. Very good results were achieved on the co-cultivation of Rhodosporidium toruloides and Desmodesmus velitaris on a medium containing waste frying oil with a biomass growth of 10.53 g/l. The other strains were Rhodotorula mucilaginosa and Anabaena torulosa cultured on the medium with the addition of frying oil to the medium with a biomass growth of 16.328 g/l. Acknowledgements: The study was supported by projects King (Project Nr. 7746)

Keywords: Carotenogenic yeasts, Microalgae, Cyanobacteria, Carotenoids, Lipids, Waste oils

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Immobilized enzyme microreactors for hydrolysis of protein-rich fungi biomass

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Mycelium fungi are known for high content of proteins in their biomass, and with the concentration of essential amino acids to dominate, they are considered reliable vegan protein sources. It has also been found that peptides production through protein hydrolysis of fungi biomass could enhance their nutritional value. These protein hydrolysates constitute important food

and pharmaceuticals additives. Enzymatic hydrolysis is a fast, safe, low-cost and highly controlled method that can lead to the production of oligopeptides and free amino acids. Combining continuous flow biocatalysis and enzyme immobilization technologies, we have managed to develop a microreactor system that can effectively be applied for enzymatic hydrolysis of fungi biomass towards the production of valuable bioactive compounds. Trypsin, one of the most widely used proteases, was immobilized in the interior walls of a 3D printed polylactic acid (PLA) microreactor, after applying appropriate modification protocol. Optimum immobilization conditions were investigated using azocasein as a model substrate. The prepared microfluidic system was elaborated for hydrolysates production from submerged cultivation of Pleurotus Ostreatus.

Keywords: enzyme microreactors, immobilized enzymes, protein-rich fungi biomass

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Enzymatic modification of biopolymers with bioactive compounds from green marine algae extracts

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Biomass derived from green marine algae is considered a rich source of valuable nutrients and bioactive phytochemicals with applications in food, pharmaceutical and cosmeceutical industries. Especially, species of the genus Ulva, have been proved to metabolize biomolecules such as polysaccharides, lipids, and phenols, among others. Enzymes have been widely applied for the targeted modification of natural products with the ultimate goal of enhancing their biological activities. In the present work, extracts of green algae were treated with laccase from Trametes versicolor for the enzymatic modification of their bioactive compounds. The modified extracts exhibited notable biological activities, such as antioxidant and antimicrobial. Accordingly, the modified extracts were used to enzymatically functionalize biopolymers, such as chitosan and gelatin. The successful modification was confirmed through various spectroscopic techniques, while the modified polymers exhibited enhanced biological activities compared to the non-modified ones.

This research was 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 'Aquaculture' - 'Industrial Materials' - 'Open Innovation In Culture' (project: Biomalga, project code: Τ6ΥΒΠ-00033).

Keywords: enzymatic modification, laccase, biopolymers, ulva sp., bioactive compounds

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The technologies for ultra-high throughput screening of activated cytotoxic lymphocytes

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The aim of the study is to develop a technology for ultra-high-throughput screening of activated cytotoxic lymphocytes from inflammatory foci and to determine the structure of cell receptors associated with a high level of cytotoxic activity. Objects of study: cytotoxic lymphocytes, natural killers, microfluidic technology for screening cytotoxic activity. The microfluidic technology for ultra-high-throughput screening of cytotoxic lymphocytes was used to isolate populations of CTL and NK cells for subsequent determination of the structure of the corresponding receptors. The 18 most represented TCR receptors have been identified. The TCRTRBV29-01/TRBJ01-05, TCRTRBV29-01/TRBJ02-05, and TCRTRBV13-02/TRBJ02-07 receptors were selected as the most likely candidates for the creation of lymphocytes based on them, modified with selected tumor-specific TCRs using CAR technology. The study was performed in frame of Grant of the Ministry of Science and Higher Education of Russia #№ 075-15-2020-0773

Keywords: ultrahigh-throughput screening, cytotoxic lymphocytes, granzymes

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Comparative in vivo study of a retinoic acid stent with absorbable polymer

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Novel retinoic acid eluting stents with absorbable polymer were evaluated in vivo in comparison with everolimus eluting stents and bare metal stents. Stents were implanted in the iliac arteries of 30 rabbits. 4 weeks after implantation the stents were explanted and evaluated with histology and morphometry methods. Retinoic acid eluting stents did not cause thrombus formation or excessive inflammation and had intimal thickness and rate of stenosis non statistically significantly different than everolimus eluting stents. Retinoic acid eluting stents were

found to be safe in this animal model. Retinoic acid, a non-cytotoxic compound naturally occurring in the body, can be an alternative drug for drug eluting stents.

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 (project code: T1EDK-03965)

Keywords: retinoic acid, vitamin A, stents, drug eluting stents, absorbable polymers