ORAL PRESENTATION ABSTRACTS

DOI: 10.2478/ebtj-2025-0003

Bioconversion of anti-nutritional substances in amaranth green mass

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Currently, increasing the nutritional value of compound feeds through the use of non-traditional feed crops is an urgent problem. Among the actively researched raw material sources rich in protein, as well as a number of other biologically active substances, amaranth should be highlighted. However, along with nutritional substances, the green mass of amaranth may contain anti-nutrient components that help reduce the digestibility of feed. We have established that in the green mass of amaranth, variety Giant (harvest 2023), tannins (0.42%), phytic (2.00%) and oxalic (7.20%) acids, nitrates (0.79%) accumulate, causing negative impact on the animal body. We have proposed an enzymatic method for the destruction of amaranth phytates using the preparation "Kemzyme Plus P dry". It was established that the processing of its green mass at a hydromodulus of 1:10, pH of the mixture 6.4±0.01, temperature 39±1 °C for 90 minutes with a multi-enzyme complex at a dosage of 2.5% ensured not only the complete removal of phytates and nitrates from vegetable raw materials, but also a decrease in the content of "crude fiber" by 1.4 times. This opens up the prospect of introducing more protein-grass meal from the green mass of amaranth into the quail diet and, as a result, reducing the cost of feed.
The work was carried out within the framework of the Russian Science Foundation grant no. 22-76-00062.

Keywords: amaranth, enzymatic catalysis, anti-nutritional substances

DOI: 10.2478/ebtj-2025-0003

Optimisation of multi-enzymatic BVMO-based ε-caprolactone production using viable whole Escherichia coli cells

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Introduction: Enzyme-mediated biooxidations have become an interesting toll for industrial bioprocesses and thus are holding the promise to enable a future of reduced environmental impact and more efficient production. A number of enzymatic cascade systems, in particular from the group of Baeyer–Villiger monooxygenases (BVMOs), have been reported to maximise the product yield [1-3].
Aim: This work was aimed at optimisation of ε-caprolactone production, a precursor to nylon-6, using Escherichia coli BL21(DE3) cells expressing enzymes alcohol dehydrogenase, enoate reductase, and cyclohexanone monooxygenase.
Methods and Findings: An optimisation of medium composition, induction and enzyme reaction conditions has been performed with satisfactory results. Samples from cultivations and enzyme reactions were analysed using gas and liquid chromatography techniques as well as advanced multi-modal optical imaging method.
Conclusion: The optimised protocol is planned to be used in the design of advanced electrochemical 2D nanomaterial-enabled biosensors for monitoring of Baeyer–Villiger biotransformations.
This work was supported by the Slovak Research and Development Agency under the contract no. APVV-20-0272, VEGA 1/0515/22 and VEGA 2/0164/24.

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DOI: 10.2478/ebtj-2025-0003

Self-replicating viral vectors for the production of recombinant vaccines in plants

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Plants may become promising biofactories for large-scale production of recombinant proteins due to the low cost and safety of the products. We constructed a self-replicating vector pEff based on genetic elements of potato virus X and showed that it can be used for transient expression of recombinant proteins in Nicotiana benthamiana plants with high yield, up to 1 mg/g fresh leaf tissue. Using this expression system we obtained plant-produced vaccine candidates against various infections, including influenza A and B viruses, SARS- CoV-

2 and hepatitis E virus. The immunogenicity of the antigens, especially of short peptides, was enhanced by their fusion with carriers such as virus-like particles formed by viral capsids or an artificial self-assembling peptides. Bacterial flagellin, acting as a powerful mucosal adjuvant, also was used as a carrier for antigens. Some of plant-produced vaccine candidates, particularly vaccines against influenza A based on M2e peptide and conserved fragments of hemagglutinin, have shown their efficacy in animal models. Transient expression systems based on self-replicating viral vectors have significant potential for the production of vaccines and other medically-important proteins in plants.

This research was funded by the Ministry of Science and Higher Education of Russia (project 075-15-2022-318, "Agrotechnologies for the future").

DOI: 10.2478/ebtj-2025-0003

Trash to treasure - sustainable production of bacterial cellulose from waste and its potential application in agriculture

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Bacterial cellulose (BC) is a polymer produced by Gluconacetobacter xylinus. It has many unique structural properties allowing for applications in drug delivery, wound treatment, food packaging, and agriculture. The main limitation in large-scale application of BC is the high production cost. This study aimed at low-cost and sustainable production of BC. Household waste, including fruit/vegetable peels, tea/coffee waste, and brewery industry waste were used for BC production. Waste material was subjected to hydrolysis to obtain extracts that were used as production media. All extracts were fully analysed. BC production was checked in terms of yield and thickness of the films. All BC films were studied using Fourier-transform infrared spectroscopy, Scanning Electron Microscopy, Thermal Gravimetric Analysis, and tensile strength analysis. Highest yield was obtained by standardizing parameters including medium composition, pH, temperature, time, and inoculum size. Best results were obtained using potato peel hydrolysate, with the addition of brewer's yeast at pH 6, and 30°C temperature. Although yield from potato peels was lower (133.7g/L) than that obtained using synthetic, standard medium (204g/L), the study shows the potential use of potato peels for BC production at a large scale. This approach allows for waste valorisation and is a step forward for sustainable agricultural systems.

DOI: 10.2478/ebtj-2025-0003

Determination of coagulase-positive *staphylococcus aureus* in white cheese and evaluation of their antibiotic resistance

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This study aims to investigate the presence of coagulase-positive *Staphylococcus aureus* in white cheese and evaluate their antibiotic resistance profiles. Understanding these factors is crucial for developing effective strategies to ensure food safety and address antibiotic resistance concerns in the dairy industry. In this study, 384 white cheese samples were collected from various cities across Türkiye. Typical colonies were identified in 276 of the cheese samples. Biochemical and molecular analyses revealed 85 isolates as *S. aureus.* Additionally, the presence of the nuc gene region, specific to this strain, was identified in all 85 isolates. The prevalence of *S. aureus* in the white cheese samples was determined to be 22.14%. The antibiotic resistance levels of the 85 *S. aureus* strains were assessed using the disc diffusion method. The resistance rates were as follows: 85% to penicillin G, 73% to ampicillin, 64% to oxacillin, 52% to cefoxitin, 32% to gentamicin, 28% to streptomycin, 21% to rifampicin, 19% to vancomycin, 15% to cefazolin, 9% to netilmicin, 6% to enrofloxacin, 6% to tetracycline, 5% to ciprofloxacin, 2% to azithromycin, and 2% to trimethoprim. No resistance was detected for doxycycline and chloramphenicol. The overall multiple antibiotic resistance level was found to be 75.29%.

DOI: 10.2478/ebtj-2025-0003

Networking salt ınducible kinase 1-transcription factor-microRNA regulatory perturbations on type 2 diabetes- Breast cancer co-morbidity associated molecular bridge

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Type 2 diabetes mellitus (T2DM) is associated with a moderately elevated risk of breast cancer (BC). However, the underlying molecular mechanisms are yet to be fully understood. T2DM and BC are multifactorial and polygenic in nature, hence it is plausible an interplay between various signalling pathways be wired into the co-morbidity program. Salt inducible kinase 1 (SIK1) is a hub gene previously validated in silico for T2DM-BC crosstalk. To probe into its functional niche within the co-diseasome, this study constructed a SIK1 associated regulome subjected to network modelling. Transcription factors (TF), microRNA (miRNA), hub proteins and gene mutations associated with SIK1 and its interactions were extracted from EnrichR and MuTarget, respectively. TF-miRNA regulatory network iteration was studied on Cytoscape, to identify SIK1 associated 143 protein-protein interactions (PPIs). Interestingly, these were enriched for KEGG pathways PI3K-AKT signalling, and transcriptional mis-regulations and miRNA in cancer. Furthermore, ClinVar disease terms particularly included T2DM and BC, highlighting their potential implication in co-morbidity. Top hub genes included TP53, EP300, AKT1, CREB1, HIF1A, EGFR, SMARCA4, HDAC2, NFKB1 and HDAC5. Prospective studies on potentiating these hub genes particularly in context to SIK1 molecular dynamics may provide further insights into the molecular links tying T2DM to BC.

DOI: 10.2478/ebtj-2025-0003

Nodule-forming bacteria effects on *Lablab purpureus* responses to abiotic stress

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Lablab purpureus (L.) Sweet, a legume cultivated in tropical regions, is used for human consumption and fodder. The plants, with an optimal temperature range of 18-30ºC, are generally tolerant to drought and low temperatures and establish symbiosis with soil nodule- forming bacteria. With increasing droughts, global temperatures and soil salinity, climate change severely threatens agriculture. Studying plants' responses to abiotic stress and their interactions with microorganisms is crucial for agronomic planning and enhancing crop resilience. In this work, we inoculated *L. purpureus* seedlings with wild strains of nodule-forming bacteria tolerating high NaCl concentrations, from two hosts and geographical locations: *Coronilla Juncea,* Sierra Calderona, and *Lotus creticus*, La Albufera Natural Park, Valencia. Seedlings were subjected to 150 mM and 300 mM NaCl and water stress, alongside a well-irrigated control. After 17 days, morphological, phenological, and biochemical stress markers were analysed. Salt stress hindered plant growth, induced proline accumulation, blocked Na^+ transport at the root level and altered other ion concentrations, whereas water stress curbed root parameters and enhanced glutathione reductase activity. Under stress, bacteria produced heterogeneous effects on leaf and root growth, and on leaf Cl^- and root K^+ contents.

DOI: 10.2478/ebtj-2025-0003

Personalised biosynthetic advanced hydrogels as dressings for chronic wound management

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Wound healing is a complex process aimed at restoring tissue integrity. Acute wounds can become chronic without proper care, and standard over-the-counter dressings often lack the versatility needed for different wound types. Effective wound management requires personalised care, tailored to the patient's specific pathology and wound characteristics. This project focuses on creating custom-shaped and sized dressings to meet individual needs, representing a novel approach in wound management.

Hydrogels are promising candidates for advanced moist wound dressing material. Bacterial cellulose (BC) is a biosynthetic hydrogel material that is produced by several bacterial genera including, Komagataeibacter xylinus. This study investigates the production and analysis of BC for customised wound treatment, focusing on factors such as dimensions, thickness, size, and shape. Additionally, it provides a detailed overview of BC loaded with different active phytomedical healing agents.

This talk will offer insights into the biosynthesis of BC and its physicochemical properties as a wound dressing matrix. All hydrogels were characterised based on their potential wound dressing applications, with in vitro tests conducted to evaluate their properties like, but not limited to, antimicrobial activity, hemocompatibility, cytocompatibility, and light transmission characteristics.

DOI: 10.2478/ebtj-2025-0003

Evaluation of bio-anode performance in a microbial electrolysis cell fed with waste organic feedstock

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Bioelectrochemical systems (BES) employ solid-state electrodes to stimulate microbial metabolisms. Among BES, methane-producing microbial electrolysis cells (MEC) represent a promising technology for the biological upgrading of biogas, a gas mixture mainly composed of CH_4 (50 - 70 %, v/v) and CO_2 (30 - 50%, v/v). In a CH_4-producing MEC, methanogens drive the cathodic CO_2 reduction into CH_4, with the required energy input partially provided by the biologically-driven oxidation of organic (waste) substrates at the MEC bio-anode. However, few literature studies investigated the bio-anode performance using real feedstocks. In this research, the bio-anode ability to oxide a real feedstock was evaluated in terms of current generation, COD (Chemical Oxygen Demand) removal efficiency and Coulombic Efficiency. The MEC was operated with the anode polarized @+0.20 vs SHE (Standard Hydrogen Electrode) to allow the electroactive biofilm formation. It was continuously fed with the liquid fraction of the effluent deriving from an anaerobic fermenter treating food waste made of ~57% of organic acids with respect to the overall COD. The generated current resulted in the production of reduced compounds, i.e. H_2, CH_4, at the MEC cathode with a production rate of ~30 mEq/d and ~11 mEq/d, respectively.

DOI: 10.2478/ebtj-2025-0003

Two stages process for bio-hydrogen production from cheese whey: coupling dark fermentation and microbial electrolysis cell

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Microbial Electrolysis Cells (MECs) attracted significant interest for their ability to combine waste treatment with bio-hydrogen (H_2) production. We investigated MEC as a cascade process integrated with Dark Fermentation (DF) to enhance the overall H_2 recovery from Cheese Whey (CW). CW fermentation with indigenous microflora, using tailorized conditions, accounted for H_2 production of 282±50ml H_2/L, whith a yield of 42±8ml H_2/gVS. MEC experiments were conducted in H-cells. Before feeding with fermentation effluent, two enrichment strategies of Electro-Active Biofilms (EABfs) from activated sludge were tested, using different substrates (i.e. acetate and synthetic mixture of carboxylic acids). EABfs acclimatized on acetate and synthetic mixture showed similar performances in terms of CE (113±1 % acetate and 130±18% mixture), %CCE (25±1% acetate and 26.5±2.5% mixture) and hydrogen production (1.7± 0.8 meqacetate and 3.2±0.7meqmix). Then, the H-cell were fed with DF effluent (33% lactate, 12% propionic acid ,3% acetic acid) and EABfs response was evaluated. CCE obtained with biofilm acclimatised on acetate and synthetic mixture were 66±22% and 83±15% respectively. H_2 production was 1.26±0.2meq and 5.73±2.4meq, with biofilm acclimatised on acetate and synthetic mixture, respectively. Thus, the enrichment on synthetic mixture resulted in the development of a more effective biofilm, able to convert DF effluent more efficiently.

DOI: 10.2478/ebtj-2025-0003

Study of new synthesized piperazine compounds as a new leucopoiesis- and immune-stimulating drugs

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The need of the pharmaceutical market for new leukopoiesis-stimulating drugs and drugs that stimulate immunity is wide. The stimulus for the search for a new effective leucopoiesis- and immune-stimulating drug among compounds with piperazine nuclear was the manifestation of a wide spectrum of activity by the drug Polyoxidonium. The drug has a wide range of pharmacological effects: leukopoiesis-stimulating, immune-stimulating, detoxifying, antioxidant and membrane-protective. The study received 4 compounds under the code BSD: BSD-56, BSD-57, BSD-58, BSD-59. All compounds have piperazine nuclear and several types radicals. For the research 56 adult laboratory albino rats of 12- 18 weeks of age weighing 210-280 g were used for research. The studies were carried out in accordance with the "Rules for conducting preclinical (non-clinical) studies of biologically active substances" (2020). The leucopoiesis- and immune-stimulating activity of the compounds was carried out on a model of cyclophosphamide-inducing pancytopenia. The BSD-59 compound had a high leukopoiesis, erythropoiesis, and thrombopoiesis-stimulating activity, exceeding the activity of the reference drug Levamisole. The BSD-59 compound strongly stimulated intracellular microbicidal activity, the efficiency of polynuclear enzyme systems, and potentiated the adhesive properties of phagocytic cells. As a result of research the new chemical series of compounds is promising for the search.

DOI: 10.2478/ebtj-2025-0003

Improvement of Bacilysin Production in Bacillus subtilis by CRISPR/Cas9-mediated editing of the 5'-untranslated region of the bac operon

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Bacilysin is a dipeptide antibiotic composed of L-alanine and L-anticapsin produced by certain strains of B. subtilis. Due to its potent antagonistic effects on various bacterial, fungal, and algal pathogens, bacilysin is gaining increasing attention in industrial agriculture and pharmaceutical industries. However, its use in industrial applications is hindered by its low production in the native producer. The biosynthesis of bacilysin is mainly based on the bacABCDEF operon. Examination of the sequence surrounding the upstream of the bac operon did not reveal a clear, strong ribosome binding site (RBS). Therefore, in this study, we aimed to investigate the impact of RBS as a potential route to improve bacilysin production. For this, the 5' untranslated region (5'UTR) of the bac operon was edited using the CRISPR/Cas9 approach by introducing a strong ribosome binding sequence carrying the canonical Shine-Dalgarno sequence (TAAGGAGG) with an 8 nt spacing from the AUG start codon. Strong RBS substitution resulted in a 2.87-fold increase in bacilysin production without affecting growth. Strong RBS substitution also improved the mRNA stability of the bac operon. All these data revealed that extensive RBS engineering is a promising key option for enhancing bacilysin production in its native producers.

DOI: 10.2478/ebtj-2025-0003

Reinventing pleasure foods for Sustainable Healthy Diets

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Sustainable healthy diets are key for tackling the growing prevalence of dietary related diseases, improving public health and meeting consumer demand for healthier options while supporting healthier planet. However, pleasure foods such cookies and chocolates generally contain ingredients that can cause adverse reactions to certain people and are known as rich in unhealthy ingredients such as saturated fat and added sugars. On the other hands, they are convenient food with a long shelf life and largely consumed by most of the population, making them a good matrix for micronutrients fortification and addition of other bioactive health promoting compounds.
Based on these principles, we have harnessed formulation engineering, ingredients substitution and fortification to develop no added sugar chocolate spreads fortified with vitamin D, magnesium and calcium and gluten free biscuits showing medium or low glycemic index. Therefore, they could be consumed by both people with diabetes and gluten intolerance.
The presentation will highlight our approaches from physico-chemical, sensory acceptability and inclination for buying the developed products to in vitro and in vivo testing with human subjects. Some of the formulation and processing challenges will also be discussed alongside future research directions.

DOI: 10.2478/ebtj-2025-0003

Physiological effects of an electric field and Mg^{2+} on Streptomyces lividans TK24 during the productions of novel antimicrobial compounds

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Combining biological and electrochemical methods has proven to be a promising tool in manipulating the microbial physiology, with improved performance in obtaining molecules of interest reported. However, so that this technology can be applied on an industrial scale, it is essential to understand the effects of an external electric field (EF) at the cellular level. The present work shows an evaluation of the physiological effects of a low-intensity electric field (2 V/cm,10 h) applied to a Streptomyces lividans TK24 liquid medium culture (conformed by glycerol, bacto tryptone, and magnesium sulfate). *S. lividans* TK24 is a strain known for produce antimicrobials and other interesting metabolites. It was observed that when the EF was applied in the growth phase, antimicrobial activity was inhibited, while carbohydrate and stored lipid production was increased. When the electric field was applied in the stationary phase, the antimicrobial activity and the production of actinorhodin were improved. The addition of Mg^{2+} to the culture medium promoted actinorhodin production, and the antimicrobial production times decreased by 62.5%, while zeta potential of cells was modified by Mg^{2+} and the electric field.

DOI: 10.2478/ebtj-2025-0003

A molecular insight into the interactions of astaxanthin with human hepatic and colorectal cancer cell lines

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Astaxanthin ($C_{40}H_{52}O_4$) is a well-known natural carotenoid produced by several members of microalgae, fungi and bacteria. Astaxanthin shows valuable biological features due to the presence of hydroxyl and ketone functional groups in its molecular structure and it is widely used as food supplement and studied as bioactive drug. In this regard, the present study was conducted to investigate medicinal potential of Astaxanthin against HT-29 and HepG2 cancer cell lines using in silico molecular docking technique. The 3D molecular structure of Astaxanthin (CID:5281224) was obtained from PubChem, and the target receptors mTOR (7PED), CDK2 (8BYA), CDK4 (6WW8), JNK1 (2NO3), JNK2 (7CML), JNK3 (4H36) and Bcl2-xL (2W3L) from RCSB Protein Data Bank. The preparation of ligand and receptors for docking was done by using LigPrep, Protein Preparation Workflow and Receptor Grid Generation tools of Schrödinger. Then, docking studies were performed by Schrödinger Glide and visualized by Schrödinger Maestro version 13.9.138. The results showed that Astaxanthin have a binding affinity for 7 targets and scores (kcal/mol) were -4.764 for mTOR, -5.561 for CDK2, -4.145 for CDK4, -3.960 for JNK1, -4.180 for JNK2, -4.226 for JNK3. Thus, it was concluded that Astaxanthin have significant potential for development of new cancer treatment strategies.

DOI: 10.2478/ebtj-2025-0003

Designing a pre-analytical portable reader for on-site aflatoxin detection

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Aflatoxins are a global public health issue that causes serious health and economic consequences. Aflatoxin's chemical and structural characteristics have led to the development of instruments for measuring and identifying its presence in food. This study investigated spectral-based sensor systems for aflatoxin detection and discussed their use as a pre-analytical in situ detection method. A device prototype was manufactured using a 3D printer. Different amounts of the contaminated pistachio sample obtained from the National Reference Food Laboratory

were exposed to UV-A light, and its fluorescence properties were observed on UV-VIS Spectrometer, AS7262, ISL29125 components. In spectrometer studies, the aflatoxin sample gave a maximum peak between 440-560 nm and 480-510 nm. Green and blue fluorescence readings were provided in AS7262 sensor-based studies, and blue fluorescence readings were provided in ISL29125 sensor-based studies. Significant differences were obtained between the readings of both sensors. The values obtained from two different sensor systems have been compared statistically. The spectrometer results have been used to control sensor-based systems' accuracy. The AS7262 sensor-based system provides more accurate results than the ISL29125-based system. It was decided that the pre-analytical portable reader design for on-site aflatoxin detection could be used as a detection method.

DOI: 10.2478/ebtj-2025-0003

Chromosomal microarray on product of conception in early pregnancy loss: A case report

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Introduction: Chromosome abnormalities are estimated to be the cause of 15% to 60% of spontaneous abortions or may result in malformed fetuses or neonatal deaths. Analysis of fetal tissue / products of conception (POC) by Chromosomal Microarray (CMA) may identify the cause of the spontaneous abortion, malformation or neonatal death.

Case report: The patient, 32 years old and a recent pregnancy loss. She and her partner have been trying for a pregnancy for past 12 months but have suffered from two miscarriages between 6 and 9 weeks' gestation. The CMA analysis of her last miscarriage fetal tissue showed 16.9 Mb deletion of X chromosome (chrX: 21226521-38172449x1). The deleted region contained 43 OMIM genes. Both of the parents are phenotypically healthy and have normal karyotype.

Material Metod: Genomic DNA samples were extracted with the Gentra Puregene Kit. CMA was performed using the CytoScan 750K array according to the manufacturer's instruction. All data were visualized and analyzed with the Chromosome Analysis Suite software.

Conclusions: CMA may also help in assessing risk for additional pregnancy loss or having subsequent children with chromosome abnormalities, and may be important in managing future pregnancies.

Keywords: Chromosomal Microarray, Products of conception, Chromosome.

DOI: 10.2478/ebtj-2025-0003

Lutexin as a potential inhibitor for Monkeypox Virus

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Lutexin is a derivative of luteolin that is a natural flavonoid that has protective and therapeutic features against various types of viral infections. However, its effects against Monkeypox Virus, recognized as a recent potential pandemic agent that cause mpox viral illness, is still unknown. Thus, the present the present study was conducted to determine protective and therapeutic features of Lutexin against Mpox using molecular docking approach. The 3D lutexin structure (ZINC4963990) was obtained from ZINC database, and the Mpox-related target receptors A41L_protein (8JC6), poxin (8C9K), MPXV_A7_protein (8IZU), D5_ADP (8HWD), AEP_domain_of_MPXV_E5 (8XIG), D5_ADP-ssDNA (8HWF), VP39_transferase (8OIV), DNA_replication_holoenzyme_complex (8J8F), methyltransferase_VP39 (8CEV) and polymerase_complex_F8-A22-E4-H5 (8WPE) from RCSB Protein Data Bank. The ligand and receptors were prepared for docking by using LigPrep, Protein Preparation Workflow and Receptor Grid Generation tools of Schrödinger. Then, docking studies were done by Schrödinger Glide and the results visualized by Schrödinger Maestro version 13.9.138. The docking results showed that Lutexin have a significant binding potential for 10 targets and scores varied from -3.885 (8IZU) to -8.346 (8HWD) kcal/mol. As a consequence, the results of the present study showed protective and therapeutic potential of Lutexin on Mpox outbreak. They are considered as valuable for development of new biotechnological products.

DOI: 10.2478/ebtj-2025-0003

Authentication of oil plants in foods using novel PCR approaches

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Sunflower (Helianthus annuus), soybean (Glycine max), maize (Zea mays), sunflower (Helianthus annuus) and rapeseed (Brassica napus) are important oil plants. They are widely used in the food and biodiesel industries. Effective detection of these plants is important for safe food and high quality biodiesel production. This study presents new multiplex and nested polymerase chain reaction (PCR) approaches for the reliable detection of these plants in foods and oils. The work included: design of PCR primers, genomic DNA extraction; development of PCR systems; evaluation of genomic DNAs and PCR products by agarose gel electrophoresis. Seeds, flours, and various proces-

sed foods, including cold-pressed and refined cooking oils, were examined. Efficient nested and double PCRs targeting species-specific genes such as sunflower helianthinin, soybean lectin, maize zein, as well as rapeseed acetyl-CoA carboxylase were developed. Novel fourplex PCR allowed simultaneous identification of sunflower, soybean, maize, and rapeseed. Testing of various food products has shown that new PCR methods are useful for accurate, fast and cheap detection of oilseeds in foods and oils.

Acknowledgments This work was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG) [Grant AR-22- 636].

DOI: 10.2478/ebtj-2025-0003

Evaluation of the permeability of epithelial barriers treated with dietary supplements in different in vitro models

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The ability of stress to influence the permeability of epithelial barriers has been demonstrated. Psychosocial stressors can modify the integrity of the epithelial barrier. Being able to maintain and restore the integrity of epithelial barriers is an important therapeutic goal. Due to their health-promoting properties, there is growing interest in the use of functional foods, dietary supplements and pharmaceutical formulations. The aim of this study was to analyze the effects of vitamin D on his ability to re-establish the resistance of epithelial barriers when insulted with ethanol or lipopolysaccharide (LPS). Transepithelial/transendothelial electrical resistance (TEER) was used. TEER is a widely accepted quantitative and conservative technique to continuously monitor barrier integrity and the strength of junctional proteins during the various phases of growth and differentiation of epithelial and endothelial monolayers. Vitamin D has been evaluated in a variety of epithelial barrier models, including colon adenocarcinoma cell lines (HT-29, Caco-2, SW-480), cancer gastric NCI-N87 cell line, and epithelial hepatic carcinoma Hep-G2 cell line, before and after application of ethanol or LPS. The results showed that vitamin D was able to prevent the increase in permeability caused by ethanol or LPS and, in different cell types, restore the integrity of the barrier with different timings.

DOI: 10.2478/ebtj-2025-0003

Nanofibers with antimicrobial activity as potential would healing delivery system

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Nanofiber-based delivery systems have recently been the focus of increased investigation for wound healing applications, due to their ability to administer active substances directly to the target site, thereby facilitating tissue repair and regeneration while preventing infections [1,2]. This study was focused on development of an antimicrobial delivery system utilizing PHB nanofibrous structures and subsequent incorporation of liposomes into nanomaterial mesh. The materials were enriched with phenolic components and antibiotic to enhance antimicrobial protection at the wound site. The nanostructures were fabricated by forcespinnig and a controlled environment in vitro model was established to evaluate the release of the encapsulated substances by HPLC. In addition, the materials were tested for antimicrobial activity against selected strains of microorganisms. To determinate the suitability for local applications, a cell viability assay on HaCat cell line was performed. The results indicated strong antimicrobial activity against selected bacterial strains and pathogenic fungi, while demonstrating no cytotoxic properties. The nanofiber delivery system showed promising potential for localized wound treatment.

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DOI: 10.2478/ebtj-2025-0003

Cellular Effects of Propranolol and Labetalol on Parental and Doxorubicin-Resistant Breast Cancer Cells

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Propranolol, a first-generation non-selective beta-adrenergic receptor (β-AR) antagonist (beta-blocker), has potential anti-cancer properties. Cancer cells with a multidrug resistance (MDR) phenotype may resist multiple drugs due to various cellular protection mechanisms. The aim of this study is to investigate the effects on propranolol and labetalol (α1/β-AR antagonist) comparatively on parental (MCF-7/S) and doxorubicin-resistant MCF-7 (MCF-7/Dox) breast cancer cell lines for in vitro demonstration of repositioning potential of beta-blockers. The cytotoxicity of propranolol and labetalol on MCF-7/S and MCF-7/Dox cells was determined using MTT assays at 48 and 72 hours. Flow cytometry analyzed cell cycle and apoptosis induction, and the colony-forming potential and migration

capacity were tested. Labetalol and propranolol caused concentration-dependent reductions in MCF-7/S and MCF7/Dox proliferation, with labetalol showing higher cytotoxicity at 72 hours. MCF-7/Dox cells showed cross-resistance to both drugs. Both drugs notably reduced the colony-forming capacity of both cell lines, with propranolol inducing a lesser reduction in MCF-7/Dox migration. Both drugs caused G2 arrest in MCF-7/Dox cells and increased G1 phase in MCF-7/S, and induced apoptosis. Further studies will evaluate the effects of these beta-blockers on the transcriptomic profile of MCF-7/S and MCF-7/Dox cells. Understanding these effects could contribute to repositioning beta-blockers in cancer treatment strategies.

DOI: 10.2478/ebtj-2025-0003

Alteration of Mir-502-3p and Mir-101-3 and the NHEJ pathway in DNA damage response of doxorubicin-resistant breast carcinoma cells

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Non-homologous end joining (NHEJ) repair pathway, has been associated with multidrug resistance. It is known that the expressions of various miRNAs associated with repair are altered in drug resistance. The aim of this study was to investigate the role of Mir-502-3p and Mir-101-3p in DNA repair and the involvement of NHEJ pathway in damage response of doxorubicin-resistant cells.

miRNAs, which are known to play a role in apoptosis, cell cycle regulation, and damage response, were analyzed in-silico, and pathway analysis was performed using the targets of Mir-502-3p and Mir-101-3p. Expressions of miRNAs were examined with qRT-PCR. Doxorubicin-induced DNA damage was tested by Comet assay. Immunofluorescent labeling of 53BP1 was performed for the analysis of NHEJ repair. Expression of the genes coding the essential components of NHEJ pathway were analyzed by qRT-PCR.

Results showed that Mir-502-3p, Mir-101-3p expressions were decreased 3-fold and 5-fold in doxorubicin-resistant cells. 53BP1 analysis showed that repair rate increased 2-fold in sensitive cells but not in MCF7/Dox. Expressions of all NHEJ- related genes decreased in resistant cells. Results suggest that the Mir-502-3p and Mir-101-3p, which are known to play role in cell cycle control and DNA damage are altered in parallel with the NHEJ pathway in drug resistance.

DOI: 10.2478/ebtj-2025-0003

Comparing long-read sequencing technologies

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Long-read sequencing produces genomic data by generating individual reads that are thousands of nucleotides or more in length. These reads typically come from "native" DNA or RNA from a biological sample which is not been amplified by PCR, preserving any base modifications present. In the rapidly advancing field of genomics, choosing the right long-read sequencing technology is key to meeting your experimental goals smoothly and successfully. It's important to understand how different long-read data types, namely HiFi reads and nanopore reads, stack up against each other based on the specific project's needs like application fit, cost, resources, and data accuracy. HiFi sequencing is your a good choice if your project needs high accuracy to identify genetic variants and mutations. HiFi sequencing combines long read lengths with exceptional accuracy, even in the challenging "dark" regions of the genome, such as GC-rich or repetitive areas. HiFi sequencing can accurately call and phase small and large variants, providing crucial haplotype information for disease research. During disease outbreaks, speed is very critical. Nanopore sequencers offer a rapid setup and real-time data generation, making it possible to identify pathogens quickly. This capability makes it a valuable tool for scientists managing infectious disease outbreaks.

DOI: 10.2478/ebtj-2025-0003

Unveiling the genetic diversity of cerebral palsy mimics using exome sequencing in the Turkish cohort

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Background: Cerebral palsy (CP) is a group of disorders with non-degenerative motor function deficits. Regardless of risk factors, CP is linked to an underlying genetic cause, despite its unclear origins. Our approach aims to enhance diagnostic precision by using trio- WES data and reanalyzing CES and WES data from unresolved cases in the Turkish cohort.

Methods: Unsolved cases with CP and CP-like symptoms were selected based on criteria for CP clinical diagnosis. Biological materials and omics data were integrated into the ACU-Biobank. Genome analysis was performed using a custom-built pipeline, and candidate variants were confirmed by Sanger sequencing when necessary.

Results: Exome analysis revealed that 41% of patients carried pathogenic or likely pathogenic variants, causing, or increasing

the risk of CP. SPAST, and COL6A1 each harbor two or more candidate variants. Also, variants of uncertain significance were detected in 32% of cases within genes correlating to the observed phenotypes, and Trio-WES revealed de novo mutations in KIF1A and SYNGAP1 genes.
Conclusions: These findings highlight the intricate genetic landscape of CP. Further research is crucial to identify CP's genetic etiology and improve diagnostic precision.
Grant: This project was granted by TUBITAK #S221889.

DOI: 10.2478/ebtj-2025-0003

The Comparison of Interleuken-12 (IL-12) Gene Expression Analysis in The Use of Cold Atmospheric Nitric Oxide (NO) Gas and/or with NPH Insulin Cream in Healing Wounds with Tissue Loss in Diabetic Rats

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This Study was designed to compare between different treatment effect on the expression of Interleukin-12 (IL-12) gene and how play a role in healing of diabetic wounds. In this study experiment, the compound treatment contains both atmospheric Nitric Oxide treatment (NO) and NPH Insulin cream, newly discovered to have a significant effect on IL-12 and Diabetic Wound healing. The total number was 24 samples from Diabetic rats divided in these four groups diabetic control (DC), diabetic insulin (DI), diabetic nitric oxide (DNO), Diabetic Insulin and nitric oxide (DINO), and this according to the treatment applied in each group. This study did a gene expression analysis to IL-12 by used RT-PCR. By comparing between four groups: DC, DI, DNO, and DINO to get fully understand the effect of these treatment in the mRNA gene expression of IL-12. And this study found a high significant on the DINO when compared with DC with (P=0.0437). When gene expression of IL-12 was high amount, this means that there's an inflammation and be chronic wounds so need more time in healing. This study shown in DNO a low gene expression of IL-12 because there was no inflammation and NO made negative effect to IL-12.

DOI: 10.2478/ebtj-2025-0003

An in vitro visit to Extracellular Vesicles as screening tools for breast cancer

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Extracellular vesicles (EVs) contain biomolecules like oncogenes and metastasis-related proteins that reflect cancer progression. Detecting EV characteristics helps distinguish normal from pathological conditions, with a focus on breast cancer in our study. Analyzing biomolecule composition, size, and concentration offers a projection of disease status. In our aim to evaluate EVs as screening tools for the disease in question, we isolated EVs from various breast cancer, mammary epithelial, and fibroblast cell lines. We analyzed cell surface markers, sizes, and concentrations across groups to grasp their diversity in breast cancer. Our study offers optimization procedures for EV isolation and characterization while setting the stage for refining EV-based biomarkers tailored to breast cancer subtypes, potentially leading to minimally invasive diagnostic tools based on EV analysis. Using in vitro samples, our approach comprehensively assesses EVs in breast cancer, with the promise of advances in disease diagnosis and management.
This study was supported by the TÜBİTAK 1005 Program, project number 123S980.

DOI: 10.2478/ebtj-2025-0003

Caffeic acid extract induces autophagy in breast cancer cell lines by increasing oxidative stress

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Breast cancer ranks first among the most diagnosed cancer types and fifth in cancer-related deaths. Research has shown that phenolic acids have medicinal properties such as antioxidant, anti-inflammatory, immunomodulatory, and anticancer effects. Caffeic acid is a phenolic acid found in all plants. This study aims to investigate the effects of caffeic acid on intracellular autophagic oxidants, antioxidants, and cell proliferation in breast cancer cell lines.
In this study, breast cancer cell lines were used. Effect on cell proliferation was determined by MTT assay. Total oxidant and antioxidant levels were measured from cell lysates using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Autophagy was analyzed using ATG5 protein quantification via commercially available enzyme-linked immunosorbent assay (ELISA) kits and the detection and quantification of AVOs (lysosomes and autophagosomes) through immunofluorescence AO staining.

It was observed that treatment of breast cancer cell lines with caffeic acid resulted in a decrease in intracellular antioxidant levels and an increase in oxidant levels, triggering autophagy in cells along with increased oxidative stress.

DOI: 10.2478/ebtj-2025-0003

Antibiotic-resistance and dynamics of urinary tract infections after Covid-19 in Tirana region

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During the COVID-19 pandemic, the misuse of antibiotics in hospitals and outpatients was considerable, not only in Albania but also beyond. Referring to the inopportune use of antibiotics during pandemic, self-medication without a prescription and also highly recommended by family doctors, today antimicrobial resistance is a challenge. The aim of this study is to identify the effect of the COVID-19 pandemic on urine culture results and also to evaluate the antibiotics resistance or sensitivity in patients with suspected urinary tract infections. Urine samples collected in Tirana during 2022, were analyzed. The evaluation was carried out for the most frequent microorganisms, *Escherichia coli* and *Staphylococcus Aureus*. Microbial identification and Antibiotic Susceptibility Testing (AST) are performed using VITEK2 System.The wide variety of VITEK2 identification cards band and AST cards determines if the bacteria carried by each patient is sensitive (S), resistant (R) or has medium resistance (M). Antibiotics that have shown greater resistance to both microorganisms are Ampicillin, Erythromycin and Clarithromycin. Increased resistance of those antibiotics comes as a result of their increased use, especially during pandemic. Antibiotic-resistance assessment will guide in the near future more accurate treatments only when they are absolutely necessary and with doctor's prescriptions.

Keywords: antibiotics-resistance, urinary infections, *E.coli*, *S.aureus*, Tirana

DOI: 10.2478/ebtj-2025-0003

A candidate cell-free cellular therapy for non-integrated RNA virus infections

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The COVID-19 pandemic, sparked by SARS-CoV-2, is one example of an RNA virus-induced epidemic or pandemic. The RNA viruses can create a catastrophic effect on trade, travel-related interactions, social structures, and public health in the near to medium term. Diversifying preventive and therapeutic approaches are therefore essential for treating diseases caused by viruses in humans and animals. A novel strategy for fighting RNA viruses is described in the proposed study. RNA molecules are targeted and cut by the CRISPR-Cas13 system. We integrated the CRISPR-Cas13 system into a cell-free cellular therapy approach. We engineered mesenchymal stem cell- derived extracellular vesicles to carry guide RNA-Cas13 complexes. The studies in progress will determine the transportation effectiveness of the guide RNA-Cas13 complexes into cells and the success for inhibiting SARS-COV-2 viral replication. The research offers a specific inhibition of viral replication and regeneration of tissues effected by disordered immunological reactions caused by the infection.

The study (#122S590) was funded by TUBİTAK, Programs for Supporting Scientific and Technological Research Projects.

DOI: 10.2478/ebtj-2025-0003

Electrophysiological characterisation of human cumulus cells

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Cumulus cells are somatic cells which differentiate from granulosa cells, surrounding the oocyte and communicating with it through gap junction and trans-zonal projections. Hitherto, there are no studies regarding the electrophysiology of CCs. The aim of the study was to characterise the human cumulus cells from an electrophysiological point of view, using the patch-clamp technique in whole- cell dialyzed configuration, the transcriptional analysis by rt-PCR and immunocytochemistry for the localisation, on human CCs from 15 patients undergoing in vitro fertilisation. Three types of currents have been characterised biophysically and pharmacologically: a nonspecific cation current (TRP-like) blocked by barium and extracellular acidity, an inactivating voltage-dependent potassium current (IA) sensitive to 5-(4-phenoxybutoxy)psoralen (PAP-1) and a large-conductance potassium current (TEA) sensitive to low concentrations of tetraethylammonium (TEA). Furthermore, three populations were defined based on the expressions of the recorded currents: a cumulus cells type 1 (CC-type 1) composed of TRP-like and IA currents, a CC-type 2 composed predominantly of TRP-like currents and a CC-type 3 presenting all three currents. This study is necessary to understand the role of cationic currents in the pathophysiology of cumulus cells. This knowledge could allow to develop new therapeutic approaches for the treatment of human infertility.

DOI: 10.2478/ebtj-2025-0003

Vasoactive ıntestinal peptide (VIP) and VIPergic fiber alterati-

ons in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a complex disorder involving reproductive, metabolic, and neuroendocrine dysfunctions. In the study, we highlighted the role of neurotransmitters in PCOS pathophysiology with particular attention to vasoactive intestinal peptide (VIP), a neuropeptide involved in vasodilation and hormonal regulation, which may disrupt the ovarian microenvironment in PCOS, contributing to abnormal folliculogenesis. In this study, an increased level of VIP in the follicular fluid of PCOS patients undergoing in vitro fertilization (IVF) was observed, suggesting its involvement in impaired follicular development. Through histological analysis different structures within the ovary and all the follicular stages before the pre-ovulatory one were identified. Then, VIPergic fibers around these structures were examined by immunofluorescence technique. An increased nerve fiber density around follicles, blood vessels, and the ovarian stroma in PCOS patients was observed. This hyperinnervation suggested neurogenic mechanisms that exacerbate follicular dysfunction. These findings may indicate a complex interaction between the nervous system and ovarian function in PCOS, offering new perspectives on its underlying mechanisms and potential therapeutic targets. Understanding the role of neurotransmitters like VIP and nerve fiber alterations may lead to improved treatment strategies for managing PCOS symptoms.

DOI: 10.2478/ebtj-2025-0003

The periodic variability for some morphometric indices in mediterranean mussel (*M. galloprovincialis*) reared in Butrinti Lake (north-eastern coast of Ionian Sea)

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The black mussel (M. galloprovincialis) is a bivalve mollusk that is reared in a aquaculture of Butrinti Lake since the 60s of the last century. We have estimated and analyzed the average values of the morphometric variables, the allomeric relationships between them as well as the variability of the five morphometric indices for three phases of the production cycle. After the estimations about the variability index (Var %), for the average values of the five morphometric variables, we did not find a general rule to determine the changes in time of these values. Higher values of Var% were estimated for the width of the mollusk and for the posterior dorsal angle length, both in the initial phase of the production cycle. The correlation of the four morphometric parameters with the shell length and the correlation of mollusk width with the shell high was a linear equation in the form y= ax + (-) b. The values of b (y-intercept) results negative in three relationships of initial phase, one relationship of middle phase and three relationships of terminal phase of the mussel's production cycle.

DOI: 10.2478/ebtj-2025-0003

Some growth and reproduction parameters in a stock of the chub (Squalius cephalus L.,1758) from Western part of Ohrid Lake

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In this study were investigated the parameters in the VBGF and some reproductive characteristics of the chub (S. cephalus) from the Western part of Ohrid lake. The stock of chub was mainly dominated by two age-classes,2+ and 3+. The values of growth parameters in VBGF were: asymptotic length $L\infty$=35.62 cm; annual growth coefficient K=0.287/yr and the hypothetical age at which the length of the fish is zero to=-0.528 yr. The value of t50% was 1.9 yr and the value of tmax=9.92 yr. The value of growth performance index was $\Phi' = 2.561$. The average values of GSI showed an systematic increase from October to April, both for females and for males. The months April and May consists the reproduction period for the stock of the chub in the Western part of Ohrid Lake. The sex-ratio was 1.34:1.00 (F:M). The relationship between the age of the female chubs and their absolute fertility was stronger than that of the live weight (W,g) or total length (TL).

DOI: 10.2478/ebtj-2025-0003

Sphingomyelin regulates nuclear miRNA

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Nuclear sphingomyelin (SM) controls nuclear membrane and nuclear matrix fluidity, influences DNA duplication and influences RNA transcription. In specific sites of inner nuclear membrane, SM links cholesterol (Chol) to form nuclear lipid microdomains (NLMs). Here SM and CHO protect RNA from RNase digestion. To establish whether the RNAse- resistant RNA includes miRNA the miRNeasy Tissue/Cells Advanced Micro kit was used. The results showed that 22 miRna were expressed in different statistically significant manner betwe-

en NLMs and nucleui. Among these miRNA,8 showed higher expression and 14 shower lower expression than nuclei. The overexpressed miRNAs regulate synaptic reorganization, are involved in the retinoblastoma progression, promote cerebral ischemia/reperfusion injury, are involved in the glioblastoma progression and in neuropathic pain and in neuroinflammation. In conclusion, this study demonstrates that SM protects the miRNA involved in the pathophysiology of the nervous system.

Federico Fiorani and Michela Bulfoni co-first name Francesco Curcio and Elisabetta Albi co-corresponding author.

DOI: 10.2478/ebtj-2025-0003

The Effect of NOSN on the Wnt/β-Catenin Pathway Genes in MCF-7 Breast Cancer Cell Line

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Breast cancer is one of the most common cancer diagnosed in women. Traditional surgical removal techniques are still effective for many cancer types; however, innovative treatments are showing strong potential in the cancer treatment. During the last decades, fascinating researchs have been published that show the in vivo and in vitro role of O_3 in cancer cell killing while being harmless for non-cancerous cells.
The purpose of this study is to examine the effect of nanobubble ozone stored niosomes (NOSN) on ekspression levels of *APC*, *GSK3β*, *AXIN2* and *β-cathenin* genes in MCF-7. The results shows that there is a correlation between these genes expressions under different concentrations of NOSN. The similarity observed between the increase or decrease in *β- catenin* expression and the expression of other genes in MCF-7 cells with NOSN treatment suggests that β-catenin may have a different and important role in MCF-7 cells depending on NOSN.

DOI: 10.2478/ebtj-2025-0003

The vitamin D receptor gene polymorphisms: Susceptibility of thyroid disease

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Autoimmune thyroid diseases such as Hashimoto's thyroiditis and Graves' disease affects about 2% to 5% of the general population. However, women are significantly more likely to develop AITDs, with a prevalence rate of 5% to 15%, while the rate for men is much lower, around 1% to 5%. This suggests that hormonal or genetic factors may make women more susceptible to these diseases. According to the literature, there is an association between the Vitamin D receptor (VDR) polymorphism and numerous diseases, including the Autoimmune disease. Vitamin D has a key contribution to the alteration of immune regulation. As already well known The activity of vitamin D is mediated by VDR. Among the VDR gene single nucleotide polymorphisms (SNP) FokI belongs to functional SNP. Based on the literature, The association between VDR rs2228570 SNP and the risk of autoimmune thyroid disease (AITD) remained controversial. In this study, we aimed to investigate the Frequency of VDR Fokl (rs2228570) genotypes (CC, CT, TT) and alleles (C,T) among the health and thyroid disease population. The investigation of VDR Fokl (rs2228570) was conducted on 200 samples (n=100 in the control group (healthy woman) and n=100 -in the diseased (autoimmune thyroiditis)) women patients from the Georgian Population. We also analysed the association between genotype/alleles and some clinical and laboratory characteristics. The polymerase chain reaction was evaluated to examine the VDR Fokl rs2228570 SNP polymorphism. In conclusion, our study revealed that rs2228570 polymorphisms may be associated with AITD risk.

DOI: 10.2478/ebtj-2025-0003

Do soil microorganisms and plant density affect *Lotus creticus* responses to drought and high salinity?

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Lotus creticus is a legume critical for dune fixation and nutrient cycling in Mediterranean coastal habitats. Climate change threatens these ecosystems, and *L. creticus* survival must face more drastic abiotic stress and intra- and interspecific competition. The microbial coastal soil community, adapted to this harsh environment, might provide an advantage to plants in coping with abiotic and biotic stresses. This study addresses the following questions: 1) Do soil microorganisms and plant density affect the biology of *L. creticus*? and 2) What are the tolerance mechanisms of *L. creticus* against abiotic stress? To answer these questions, plants were grown individually or in groups of three in coastal soils, sterilised or non-sterilised, and

exposed to salt (100 mM and 200 mM NaCl) and water stress treatments. Plant biomass, morphometric traits, mycorrhizal structures, bacterial nodules, and biochemical markers were recorded. The results showed that plants developed better in coastal soils than in sterile soil. Plants grown in groups exhibited higher levels of osmolytes compared to those grown individually. Control plants showed better morphological development and lower stress levels than those exposed to salt and water deficit conditions.

DOI: 10.2478/ebtj-2025-0003

Up-regulation of Toll-like Receptor 4 in substantia nigra and middle temporal gyrus of patients with Parkinson's disease is associated with its colocalization with pSer129αSyn and in Iba1: implications for inflammatory response

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Neuroinflammation and immune dysfunction play a critical role in the pathophysiology of Parkinson's disease (PD) and correlates with the accumulation and aggregation of alpha-synuclein (αSyn). Toll-like receptors (TLRs) are widely distributed in the central nervous system (CNS) and expressed by the CNS-located immune or neural cells able to sense several stimuli which lead to inflammatory responses and accumulation toxic species of αSyn. αSyn can bind the TLRs and evoke an inflammatory response to clear itself and restore brain homeostasis. Therefore, the precise role of TLRs is debated as helpful and harmful effects can occur. In the present study, applying quantitative real time PCR, we found elevated levels of TLR4 in the substantia nigra (SN) and in the middle temporal gyrus (GTM) of patients with PD compared with control donors, while αSyn was downregulated, probably because of the significant depletion of dopaminergic neurons. Using multilabel immunofluorescence and confocal laser scanning microscopy, we also observed colocalization between TLR4 and pSer129-αSyn and between TLR4 and glial Iba1 in SN Lewy bodies and pyramidal neurons withinGTM compared with the same regions of the control donors. Our findings provide evidence for the up-regulation of TLR4 in PD patients and suggest a physical interaction that may evoke the activation of inflammatory response in PD patients.

DOI: 10.2478/ebtj-2025-0003

Multifunctional roles of *Bacillus thuringiensis*: Unraveling plant growth-promoting, fungicidal, and insecticidal genes through bioinformatics

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Bacillus thuringiensis (Bt) is a bacterium known for producing parasporal crystals containing insecticidal toxins, such as δ-endotoxins. In addition to its insecticidal properties, Bt plays a crucial role in agriculture by producing biologically active compounds which exhibit antibacterial, antifungal, and biofilm-inhibiting activities. Also, Bt function as plant growth-promoting rhizobacteria (PGPR), enhancing plant growth through nitrogen fixation, nutrient solubilization, phytohormone biosynthesis, and induced systemic resistance, contributing to sustainable agricultural practices. In this study, a comprehensive guide was provided on how to use bioinformatics tools to detect and analyze the genes responsible for these multifunctional roles in Bt strain SY49.1. Cry toxins were compared against the BPPRC database to assess similarities and novelty. Other toxins and virulence factors were identified using the VFanalyzer tool. Secondary metabolites were predicted, annotated, and analyzed using AntiSMASH version 4.0 and the PKS/NRPS Analysis website. The NORINE database was used to examine structural patterns in peptides of secondary metabolites. Prediction and annotation of PGPR-related genes were conducted using RAST tool. This study outlines a step-by-step approach for applying bioinformatics to identify genes responsible for Bt's insecticidal, fungicidal and PGP functions, aiding its use in sustainable agriculture.

Keywords: Bt, insecticide, fungicide, PGPR, sustainable agriculture, in silico tools.
Acknowledgement: TÜBİTAK 22AG012

DOI: 10.2478/ebtj-2025-0003

Precision medicine development at the Medical University Plovdiv

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The Medical University Plovdiv (MUP) is a leading research university in Bulgaria in the filed of molecular precision medicine and health and quality of life in green and sustainable environment. Recent development in the field of molecular precision medicine including immune imbalances and deficiencies , mutation profiling in liquid biopsy of colorectal cancer, molecular signatures and cellular metabolism in neoplastic and neurogenerative diseases, minimal disease detection by multiparametric flow cytometry, biomaterials and nanostructures for drug delivery, 3D bioprinting in oncology an regenerative medicine and targeted antibiotic therapy based on rapid microbiological diagnosis in patients with infectious disease and others will be presented. In addition, other projects such as translational and computational neuroscience, digitally guided cognition and cognitive profiling will be discussed.

The projects are funded by the Strategic Research and Innovation Program for the Development of the Medical University (SRIPD -MUP).

DOI: 10.2478/ebtj-2025-0003

The contribution of the combined use of biotechnological systems in the diagnosis of genetic diseases

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In the etiology of genetic diseases, the most frequently encountered mutation type is missense mutations, while deletions, duplications, insertions, inversions, frameshift mutations, or epigenetic changes also play a role in disease etiology. Neurofibromatosis syndrome occurs due to mutations in the NF-1 and NF2 genes. Cafe au lait spots, neurofibromas, macrocephaly, scoliosis, Lisch nodules in the eyes, learning difficulties, and an increased risk of solid tumors are observed. In order to diagnose the disease, it may be necessary to combine different methods with various technological approaches in patients with these findings. In our study, the results of 47 patients who were admitted to the clinic with a preliminary diagnosis of neurofibromatosis were evaluated. Pathogenic/probably pathogenic mutations were detected in 17 patients (36%). While the mutation in 16 patients could be detected by next-generation sequencing (10 missense, 3 deletions, 1 duplication, 1 insertion), for 1 patient (whole gene deletion), the MLPA method was required to determine the result. As shown in our study, combining NGS and MLPA systems for the diagnosis of neurofibromatosis is important for providing genetic diagnosis for a greater number of patients.

DOI: 10.2478/ebtj-2025-0003

Analyzing the antimicrobial and antiadhesive properties of secondary metabolites on Pseudomonas aeruginosa Adhesion to nylon 3D printing material: molecular docking analysis

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In recent decades, various approaches have been adopted to combat healthcare-associated infections caused by biofilms. Among these approaches is the concept of developing anti-adhesive surfaces produced by 3D printing technology, a technology that is revolutionizing the medical field by providing personalized, precise, and innovative solutions for medical diagnosis, treatment, and research. Our strategy involves the use of natural molecules to prevent bacterial adhesion and subsequent biofilm formation. For this purpose, the antibacterial activity of ten secondary metabolites was evaluated against Pseudomonas aeruginosa. The results indicate that these metabolites exhibit varying antimicrobial activity, with notable activity observed for tannic acid, gallic acid, and epicatechin gallate. Subsequently, a study on the influence of the three metabolites on the physicochemical properties of the surface of 3D-printed nylon as well as their effect on the adhesion of Pseudomonas aeruginosa was conducted. The results revealed a modification of the physicochemical properties of the material. Indeed, the treatments reduced the hydrophobic character of the nylon with a considerable increase in electron donor character. Additionally, the treatments showed anti-adhesive activity with varying percentages of inhibition. Finally, a molecular docking analysis was performed to understand the molecular interactions underlying bacterial adhesion.

DOI: 10.2478/ebtj-2025-0003

Ensuring research ıntegrity: the critical role of cell line authentication and mycoplasma testing in biotechnology

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Cell line authentication ensures the identity of cell lines, preventing erroneous results due to cross-contamination. Meanwhile, mycoplasma contamination can alter cell behavior and invalidate experimental outcomes. Despite these risks, many researchers had previously neglected such testing.

Our recent survey revealed significant gaps in routine cell line authentication and mycoplasma testing among researchers. 68% had never performed a mycoplasma test, and 95% had not conducted cell line authentication before receiving free testing service developed and validated in house (patent pending). Many researchers sourced their cell lines from local or international labs, raising the risk of contamination and misidentification.

Following the free testing, all participants found the tests valuable, with 84% willing to pay for them in the future. The tests exposed issues like misidentification and contamination, underscoring the need for regular verification.

In conclusion, routine cell line authentication and mycoplasma testing are crucial for ensuring the accuracy and reproducibility of biotechnological research. Researchers increasingly recognize their importance, but affordable access to these services is essential for maintaining high standards in cell culture work.

DOI: 10.2478/ebtj-2025-0003

SSNHL and genetic prothrombotic risk factors

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Sudden hearing loss(SHL) is defined as rapid-onset of hearing impairment in one or two ears. Hearing loss can be conductive hearing loss(CHL), sensorineural hearing loss(SNHL) or mixed hearing loss. Sudden sensorineural hearing loss(SSNHL) is a subgroup of SHL that can be consequence of abnormal functioning of the cochlea, auditary nerve or higher auditary center. The most commonly used criteria for diagnoses of SSNHL is a decrease in hearing of ≥30 decibels affecting at least three consecutive frequences. Although most of the cases are idiopathic; viral infection, ototoxic medications and ischemic events can be listed as the causes of sensorineural damage and impaired cochlear perfusion appears most important event among thoses. Cochlear perfusion can disturbed by thrombosis of labyrinth artery. For this reason we examined 20 patients with SSNHL for presence of inherited prothrombotic risk factors and 50 healthy volunteers as controls. All of subjects underwent thrombophilia panel test including MTHFR C677T and A1298C, Factor V G1691A and H1299, Factor II(prothrombine) G20210A and PAI-15G/4G genotyping. We found istatically significant association between SSNHL and MTHFR A1298C, PAI polymorhisms. These results show us that all patient with idiopathic SSNHL must be evaluated in terms of inherited prothrombotic risk factors.

DOI: 10.2478/ebtj-2025-0003

The role of a deep intronic variant in the SHH gene in the molecular pathogenesis of Dundar Acropectoral syndrome

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Dundar Acropectoral syndrome (OMIM %605967) is a rare genetic disorder characterized by syndactyly, polydactyly, and sternal anomalies. Although linkage analysis has associated this syndrome with the 7q36 region, the exact genetic mechanism remains unclear.

In this study, we performed short-read whole genome sequencing on three patients from a family with acropectoral syndrome and one healthy control. We identified the variant c.300+558_300+562delinsTTGTCAGCCA in intron 1 of SHH present in patients but absent in healthy controls. We confirmed the presence of the variant in patients by Sanger sequencing. SHH plays a crucial role in embryonic development and is associated with defects in limb and craniofacial formation.

Deep intronic variants can contribute to disease through pseudo-exon inclusion, competition with natural splice sites, disruption of transcriptional regulatory motifs and inactivation of non coding RNA genes. This study highlights the importance of deep intronic variants in genetic conditions and enhances our understanding of the role of SHH in embryonic development. Further experimental approaches, such as mini-gene assays and mouse enhancer assays, could be utilized to confirm the pathogenicity of this deep intronic variant.

DOI: 10.2478/ebtj-2025-0003

Fibulin-3 suppresses cell proliferation by regulating MYC via let-7a in SCLC

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Small cell lung cancer (SCLC) is characterized by an aggressive phenotype with a high mortality rate, early metastasis, and proliferation rate. Fibulins play a role in the creation and structure of tissues and tissue organogenesis. Also, fibulin-3 maintains and stabilizes the extracellular matrix. We focused on investigating the molecular mechanism of the effects of fibulin-3 on proliferation in SCLC. In this study, we used the fibulin-3 overexpression vector and fibulin-3 shRNA pool to check the effect of fibulin-3 expression on cellular proliferation in SCLC cell lines. Then, we used western blotting analysis to show the relationship between fibulin-3, MYC, and let-7a and used bioinformatics studies with qPCR to evaluate miRNA expressions. The effect of fibulin-3 expression on cellular proliferation and gene expression profile was evaluated in fibulin-3 overexpressing and suppressed SCLC cells. Fibulin-3 expression reduced the proliferation of N417 cells. Importantly, fibulin-3 knockdown reduced the enhanced proliferation of H82 cells. Due to the changes in Fibulin-3 expression, we evaluated the changes in proliferation and MYC that are oncogene and miRNA related to MYC. The results indicate that Fibulin-3 may regulate cell proliferation via let-7a-mediated MYC inhibition.

Keywords: SCLC, Fibulin-3, Cell Proliferation, MYC

DOI: 10.2478/ebtj-2025-0003

Artificial intelligence applications in personalised treatment approaches of genetic diseases

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Artificial intelligence (AI) has emerged as a transformative force in the field of personalized medicine, particularly in the management of genetic diseases. By leveraging advanced algorithms and machine learning techniques, AI enables the analysis of vast genetic datasets to identify disease-associated variants and predict clinical outcomes. This personalized approach to treatment offers the potential to overcome the challenges posed by the heterogeneity of genetic diseases and improve patient outcomes. Deep learning models, in particular, have demonstrated remarkable capabilities in diagnosing and treating rare and complex genetic disorders. These algorithms can efficiently

process and analyze large-scale genetic data, leading to more accurate and timely diagnoses. Moreover, AI-powered tools can assist in selecting optimal treatment regimens based on a patient's unique genetic profile, thereby enhancing the efficacy and safety of therapeutic interventions. As AI continues to evolve, its applications in personalized medicine are expected to expand further. By integrating genetic information with other clinical data, AI will provide more comprehensive and predictive models for disease management.

Keywords: AI, Personalized Medicine, Treatment

DOI: 10.2478/ebtj-2025-0003

Evaluation of genetic findings with targeted next generation sequencing in intellectual disability

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Intellectual disability (ID) is a complex neurodevelopmental disorder that causes permanent limitations in individuals' cognitive functioning and adaptive behavior. Genetic factors are one of the most important causes of ID. Both genetic and phenotype heterogeneity of ID make it more challenging for genetic and clinical diagnosis, but next-generation sequencing (NGS) technologies developed in recent years have made great progress in detecting genetic mutations and rare variants in intellectual disability, as well as identifying new pathogenic genes. It has provided important data for the discovery and potential treatment targets. This study aims to evaluate the role of previously known-associated variants obtained using the targeted NGS panel kit in our patients. Method: 85 patients (57 boys, 28 girls) who came to Erciyes University Faculty of Medicine Department of Medical Genetics with a diagnosis of Unexplained Intellectual Disability were included in the study. Patients were requested for chromosomal analysis and NGS sequencing panel analysis consisting of 198 genes for ID, respectively. The genetic analysis results of these patients were evaluated retrospectively.

Keywords: Intellectual disability, next-generation sequencing, Variant

DOI: 10.2478/ebtj-2025-0003

Prognostik significance of *HMGA2* gene methylation in ovarian cancer

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Ovarian cancer is the second most common type of gynecological cancer seen in women. In ovarian cancer the 5-year survival rate is below 50% due to late diagnosis and inadequate treatment options. The HMGA2 protein is encoded by the 160 kb long *HMGA2* gene spanning 5 exons located on the long arm of chromosome 12. It is known that it participates in the chromatin structure as a non-histone protein, which has an important structural role, as well as regulates cell proliferation. It shows an increased expression pattern in "benign" tumors and many "malignant" cancer types (ovarian, breast, pancreatic cancer, etc.). There are studies showing that the HMG2A gene has a key role in tumor metastasis. The aim of this study is to elucidate the association between the methylation pattern of the *HMGA2* gene and ovarian cancer. In this study, *HMGA2* gene promoter region methylation profile in tumor DNA was revealed via NGS. Differentially methylated regions were detected in tumor tissues of ovarian cancer cases compared to the control. A statistically significant relationship was detected between the methylation pattern of the HMGA2 and CA15-3 and overall survival. *HMGA2* gene methylation pattern has the potential to be a biomarker in determining prognosis.

DOI: 10.2478/ebtj-2025-0003

Multigene panel testing in primary immunodeficiency patients

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Primary immunodeficiency (PID) is a disease characterized by deficiencies in the innate immune system. PID involves various disorders of different components of the immune system. The genetic basis of PIDs is highly diverse. So far, more than 450 different genes have been identified as being associated with PIDs. These mutations can cause defects in cellular functions of the immune system, signaling pathways or maturation processes of immune cells. In this study, we discuss the results of 20 patients with different clinical manifestations thought to have primary immunodeficiency. Mutation analysis of PID genes (205 GEN) was performed using targeted NGS. Congenital neutropenia was diagnosed in 7/20 patients, with PGM3 (NM_001199917.2):c.61C>T in 1 of these patients and F12 (NM_000505.4):c.1079G>T in the other. Among 7 patients examined for frequent infections, 1 patient had OAS1(NM_016816.4):c.974C>A and the other patient had IL12RB1(NM_005535.3):c.1450C>T. While no variant was detected in 2 of 3 patients with a prediagnosis of hypogammaglobulinemia, PLG(NM_000301.5):c.112A>G variant was detected in 1 patient. DOCK8(NM_203447.3):c.5467C>T variant was detected in one patient examined for chronic diarrhea. No variants were detected in patients with a prediagnosis of angioedema and thrombotic microangiopathy. Genetic diagnosis in PID is of great

importance for patient follow-up and treatment.

DOI: 10.2478/ebtj-2025-0003

Select probiotics improve intestinal epithelial damage associated with obesity: In vitro analysis

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The global prevalence of obesity has tripled since 1975, and is expected to impact over half of the global population by 20351. Obesity is a chronic, relapsing, multifactorial disease and a leading risk factor for cardiovascular disease and metabolic comorbidities2. Dietary and lifestyle changes often fail to sustain weight loss, while currently available anti-obesity drugs are limited by poor safety profiles and reported side effects. Current therapies also do not address inflammation, a critical driver of obesity3. Thus, there is an urgent need for novel anti-obesity therapies with revised therapeutic targets, superior efficacy, and minimal side effects. The gut microbiota plays an imperative role in nutrient response and alterations to gut composition are well-reported in obese populations4. Targeting the gut using probiotics to manipulate the microbiome and gastrointestinal (GI) environment is an attractive option to restore microbial homeostasis and host health. Thus, this study investigates the therapeutic action of select probiotics to modulate the gut microenvironment. Briefly, Lactobacillus gasseri A237 (LgA237), L. plantarum WCFS1 (LpWCFS1), and L. fermentum NCIMB 5221 (Lf5221) were tested for adhesive, anti-oxidative and anti-inflammatory properties. All three probiotics demonstrate good adherence to IECs, indicating proper gut colonization. The probiotics also exhibit antioxidant properties via DPPH radical scavenging activity and reduced intracellular expression of reactive oxygen species (ROS) in inflamed IECs. Moreover, LgA237, LpWCFS1 and Lf5221 decrease interleukin-8 expression in lipopolysaccharide (LPS)-damaged HT-29 cells (41.19, 34.53 and 14.80%, respectively), compared to non-treated cells. Further investigation of LpWCFS1 and LgA237 revealed a significant reduction in monocyte chemotactic and activating factor (MCAF) protein expression by 63.81 and 60.33% ($p<0.0001$) in colitis-induced IECs. Overall, our results indicate excellent therapeutic potential of the tested probiotics. These findings and the use of gut-targeting therapies for for obesity and other chronic diseases will be discussed.

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DOI: 10.2478/ebtj-2025-0003

Wound dressing material on the base of cellulose 6-O- propionate -3'-aminobenzylpenicillil and carvacrol

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In the present study, immobilized on the bacterial nanocellulose (product of *Gluconacetobacter sucrofermentans* B-11267) antibiotic component and mobile antibacterial substance were used to obtain wound dressing scaffold.

We modified bacterial cellulose by the interaction with benzylpenicillin, activated by dicyclohexyl-carbodiimid with cellulose 6-O-3-aminopropionate.1,2 and 3% carvacrol was add after cellulose modification. The antibacterial activity of the composites was evaluated against two indicator microorganisms *(E. coli ATCC 25922* and *S. aureus ATCC 29213)* using the agar diffusion method.

The modified bacterial nanocellulose has shown a high antibiotic activity against *Staphylococcus aureus* and less against *Escherichia coli.* Our results indicate the crucial role of carvacrol in the antibacterial action. It may be due to the lack of ability of cellulose 6-O- propionate -3'aminobenzylpenicilli composite to permeate through the agar. Cytotoxicity test was performed on dermal fibroblasts which were stained with hematoxylin and Sudan IV, modified cellulose has shown low toxicity towards dermal fibroblast culture.

DOI: 10.2478/ebtj-2025-0003

Monitoring toxicity in sediment at the point-of-site using a dispatchable whole-cell bioluminescent bioreporter fiber-optic biosensor (SUNDANCE)

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Human activities (hydropower plants, nuclear power plants, agriculture, and transportation) oftentimes pollute our waterways and the Danube River basin is one such example, affecting both biodiversity and human health. Sediment balance changes (depostion, ersosion) finds cause in climate change and human river modulation activities (dredging, dams) and this directly affects biodiversity. Part of the complex general se-

diment quality management, and associated water quality, is to monitor its toxicity. This would enable the regulators to design a rapid actionable plan. We have designed a portable on-vessel device for the direct analysis of toxicity. This is done by using the combination of fiber-optic biosensor probes displaying at their endface entrapped luminescent bacterial bioreporters that will glow upon exposure to toxicants. These probes are inserted into a proprietary field-operable photo-detector system which enables the quantitative assessment of the overall bioavailable toxicity of either sediment or water specimens. The validated original prototype is now being reengineered to make it marine-enabled, including such features as, being rugged, durable, dispatchable, lockable, waterproof, dustproof, crushproof, light-proof, vibration protected, chemical resistant, and corrosion proof. Our first prototype, can measure a variety of toxicants, using a choice of various genetically engineered bacterial bioreporters exhibiting various sensitivities to toxicities. One problem that needs to be addressed is to try to identify the causative agent when it is in a mixture, and initial work of ours discusses the use of Dynamic Time Warping, to help solve this problem.