SYMBIOZA

INTERNATIONAL BIOTECHNOLOGY SYMPOSIUM

16-18.05.2025

Book of Abstracts

The 12th Prof. Krzysztof W. Szewczyk

International Biotechnology Symposium

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BOOK of ABSTRACTS

16–18 May 2025, Warsaw & online

The 12th Krzysztof W. Szewczyk
International Biotechnology Symposium "Symbioza"
Book of Abstracts
16–18 May 2025, Warsaw & online.
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Warsaw, 16 May 2025.

Dear Participants,

It is the 12th time we have opened the Book of Abstracts with those words. It is the 12th conference, the 12th organising committee, and 12 years of cooperation between people forming the Warsaw Society of Biotechnology 'Symbioza'. Through those years, plenty of things have changed. There were dozens of different hands working on this project and dozens of various minds, each having their ideas of how to respond to the scientific world's expectations. Yet, there is this one thing that has never changed. The idea, goal, and dream are to create a community for youth in biotech, our spare space.

A space to show the world your research, while making your first, second and further steps into the scientific world. A space to meet world-renowned scientists in person and talk to them beyond their papers. A space for networking, understanding your colleagues' work and getting a notion of the scientific landscape in biotech: in academia, industry or public administration.

Yet, this is also a safe space to share your insecurity, doubts, and burnout on our paths. While encountering different points of view, other people's stories, and already developed solutions, we come out of it more confident about our course and prepared for obstacles. Thus, this is about finding ourselves in this complex environment. We aim to keep up with the constantly changing world.

As 'Symbioza' organisers, every year we rethink the set of keynote lecturers and develop new discussion panel topics to match the front pages of scientific and non-scientific papers. We keep being open to transmitting our conference to every corner while strengthening our connections with Polish and European organisations and universities. We make it with our own voluntary hands as an independent NGO, which is our pride, to never lose the mission of doing things for students by students.

Those are your three days to listen, ask questions and meet new people over coffee and enjoy being a part of the youth biotech community. We are so happy to share those great three days with all of you!

Welcome to the 12th International Biotechnology Symposium 'Symbioza'!

On behalf of the organising committee

— **Kacper Koźluk**,
President of the Warsaw Society of Biotechnology 'Symbioza'

Honorary patronage

Minister of Science and Higher Education

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It is pointless to indicate which parts of technology or activities are more important. What is necessary, however, is mutual understanding of cooperating specialists.

— Krzysztof W. Szewczyk

Prof. Krzysztof Włodzimierz Szewczyk (1952–2011) was a remarkable scientist, and a well-recognized specialist in the fields of industrial biotechnology and bioprocess engineering. He co-founded and organized biotechnology studies at Warsaw University of Technology (WUT). He was also a director of the Interfaculty Biotechnology Centre at WUT (2007–2008) and a supervisor of the Department of Biotechnology and Bioprocess Engineering at the Faculty of Chemical and Process Engineering at WUT (2006). Since 2003 he had been a member of Committee of Biotechnology during the Presidium of Polish Academy of Sciences, the secretary of the Bioprocess Engineering section in the Committee of Chemical Process Engineering at Polish Academy of Sciences (1992–1995), a member of Programme Council of the "Biotechnology" quarter journal (2005–2010), and a Vice-President of Polish Federation of Biotechnology (2007–2010).

Prof. Szewczyk was an author of more than 120 scientific articles, co-author of 6 patents and utility designs and a co-author of 8 student handbooks. He was known as an excellent and valued teacher among students not only at his alma mater, but also at the University of Warsaw, where he taught bioprocess engineering. In 1995, he received the Silver Cross of Merit, and in 2003 he was awarded with the Commission of Education Medal and in 2008 distinguished with Ministry of Science and Higher Education Award. His colleagues, fellow professors and students remember him as an erudite, a classical music lover, and a chess enthusiast who was truly wedded to education among academic adolescents.

Sponsors

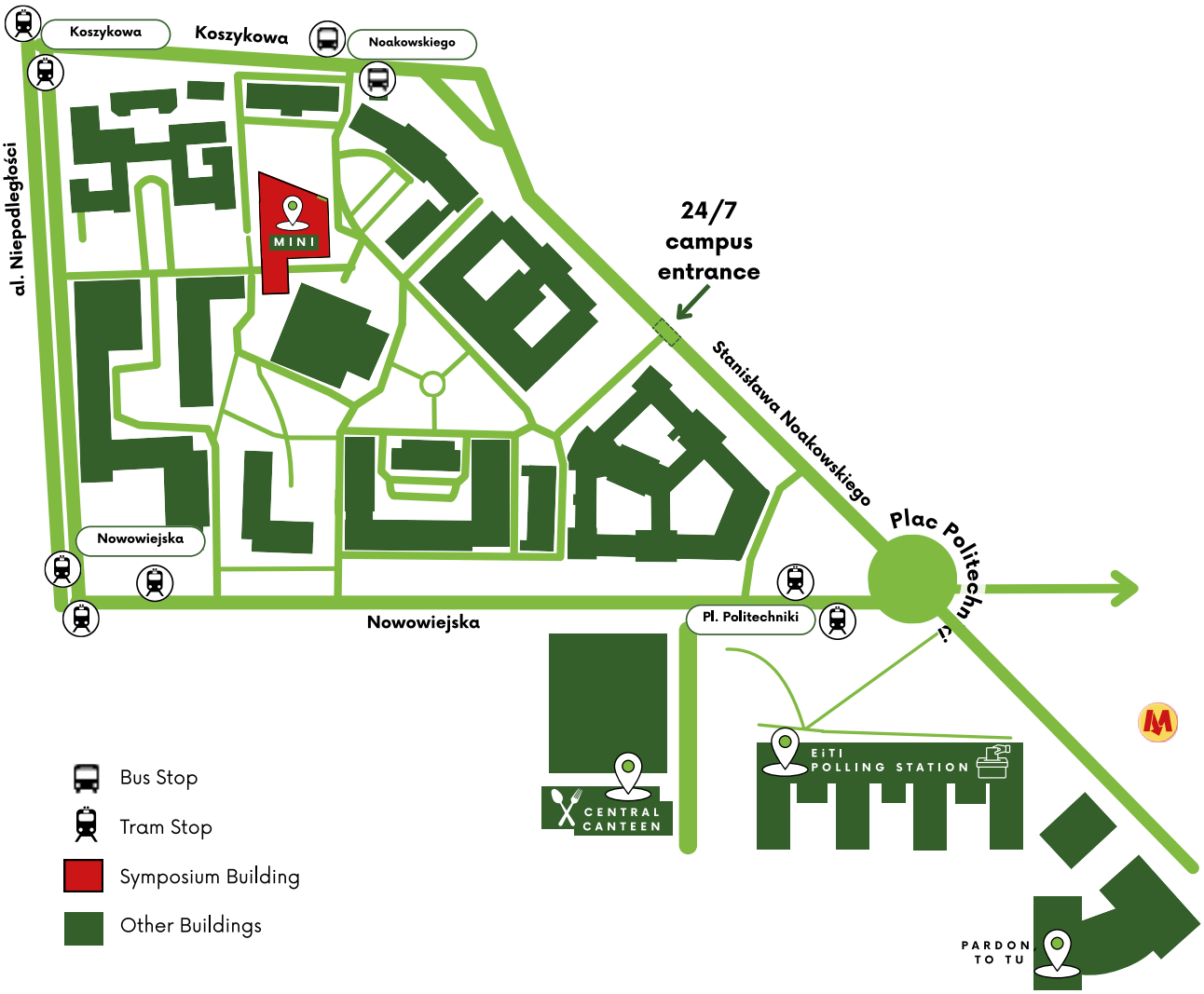
Partners

Media patronage

Conference Venue

Faculty of Mathematics and Information Science (MiNI), **Warsaw University of Technology**, established in 1999, is a leading academic unit specializing in mathematics, computer science, and data science. The faculty offers a range of undergraduate and graduate programs, including B.Sc. and M.Sc. degrees in Mathematics, Computer Science and Information Systems, and Data Science. The faculty actively participates in national and international research projects, collaborating with institutions worldwide. Research areas encompass universal algebra, differential geometry, stochastic processes, artificial intelligence, and more. Additionally, MiNI has established centres like the Center for Business Applications and the Center for Analysis and Statistical Learning to bridge academia and industry.

Campus Map



Conference Building Plan

Organiser

SYMBIOZA
WARSAW SOCIETY OF BIOTECHNOLOGY

Warsaw Society of Biotechnology "Symbioza" (WSB "Symbioza") was founded in 2013 through the collaborative efforts of students from the University of Warsaw, Warsaw University of Life Sciences, and Warsaw University of Technology. Its mission is to cultivate a platform for the exchange of knowledge and experiences among biotechnology students and researchers. At the heart of WSB "Symbioza", there is our flagship event: the Internatitonal (earlier: Intercollegiate) Biotechnology Symposium "Symbioza" (IBS "Symbioza"), which you are currently attending. Held annually from 2012, the Symposium serves as a platform for international students and PhD candidates to present their research findings and engage with peers and experts in the field. Recognized for its excellence, it was honoured as the "Conference of the Year 2019" in the prestigious StRuNa Competition.

Beyond academic conferences, WSB "Symbioza" hosts initiatives for biotechnology enthusiasts such as *Symbioza Umysłów (Symbiosis of Minds)* or *OAK Attractive Conventicles Camps*. *Symbiosis of Minds* is directed to polish high school students interested in biotechnology. *Symbiosis of Minds* mainly focuses on demonstrating that science is a unity, with all its fields interconnecting. In the current global scientific research, increasing emphasis is placed on projects that bridge different, often seemingly distant disciplines.

During *OAKs*, several days-long retreats, attendees take part in workshops that aim to improve the scientific presentation and communication techniques as well as show new ways of transferring knowledge and presenting research results. WSB "Symbioza" is also actively participating in the yearly *Science Picnic of Polish Radio and the Copernicus Science Centre* in Warsaw. During a family friendly whole day event with thousands of visitors, we strive to uncover and explain the fascinating world of biotechnology to the youngest enthusiasts.

With its diverse range of activities and commitment to promoting biotechnology awareness and education, WSB "Symbioza" continues to inspire and empower the next generation of life sciences leaders, fostering a global network of collaboration and innovation.

Organising Committee

Committee Leaders

Kacper Koźluk — President
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Dominika Krasoń — Treasurer, Head of External Communication
Paulina Dobek — Head of Logistics Section
Wojciech Garstka — Head of Scientific Section
Aleksandra Piotrowicz — Head of Promotion Section
Julia Szydłowska — Head of Technical Section

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Warsaw University of Life Sciences

Urszula Budniak, PhD
Polish Anti-Doping Laboratory, Warsaw

Iga Jancewicz, PhD
4Cell Therapies

Tomasz Kamiński, PhD
University of Warsaw

Co-organisers

The Symposium has been organised by the members of the Warsaw Society of Biotechnology "Symbioza" annually since 2013. Acknowledging its own roots, every year the Society invites life sciences-oriented student organisations of the host city to participate in shaping the event. The 2025 supporting organisers are:

Biotechnology Student Association "Herbion" — established in 2003 at the Faculty of Chemistry of Warsaw University of Technology. "Herbion" carries out a number of scientific projects, which currently include the cultivation of lactic acid bacteria on coffee waste in an air-lift bioreactor, along with studying the influence of various substrates on beer production. We also popularize biotechnology through science shows and mass events such as the Science Picnic of Polish Radio and the Copernicus Science Centre, open days at Warsaw University of Technology, and others. In addition, we organize science workshops for aspiring scientists from primary and middle schools. To help students jumpstart their scientific careers, we also take part in organizing the annual life science job fair "SSP"and help with finding projects for scientific volunteering. Other activities include educational trips and organizing monthly online lectures, known as *Meetoza – dzielimy się wiedzą*, some of which are available in English on our Facebook page.

KNBiotech Science Club — a student research organization at the Faculty of Biology and Biotechnology at the Warsaw University of Life Sciences (SGGW). Operating since 1997,the club unites students of biological faculties interested in the broadly understood biological sciences, in particular focusing on the field of biotechnology. Members carry out specific projects aimed at developing their interests. In addition to scientific activity, participants of KNBiotech are involved in popularization events, such as the Science Picnic of Polish Radio and the Copernicus Science Center, Days of Warsaw University of Life Sciences and numerous scientific conferences.

"Antidotum" — a student organization that has been active at the University of Warsaw since 2012. Over the years, the organization has become an important platform for students to explore and deepen their knowledge in the field, as well as exchange experiences with like-minded individuals. One of the most notable achievements of the organization is the successful organization of the "Biofusion" student scientific conference. This conference has been organized for seven editions so far, and has attracted significant interest from students and academics alike. Through its activities, "Antidotum" has become a hub for students interested in biomedical sciences at the University of Warsaw. Overall, "Antidotum" remains a vital and important student organization at the University of Warsaw, providing a supportive community and a platform for students to engage in meaningful discussions, exchange ideas, and explore the exciting and ever-evolving field of biomedical sciences.

The Warsaw Society of Biotechnology 'Symbioza' proudly stands in solidarity with our Ukrainian counterparts in light of the human rights violations and unjustified Russian aggression they have faced. The Organizing Committee of the Symposium is committed to actively supporting the researchers and students from Ukraine, and we are pleased to announce a special initiative to demonstrate our solidarity.

In recognition of the challenges faced by the Ukrainian society, including researchers, we declare that remote participants with Ukrainian affiliations are exempt from the conference fee. This exemption serves as a symbolic gesture, demonstrating our support and ensuring that our Ukrainian colleagues have equal opportunities to participate and contribute to the symposium. As we gather at the symposium to celebrate advancements and innovation in biotechnology, we remain mindful of the challenges faced by our colleagues in Ukraine. Through this small gesture and ongoing efforts, we strive to strengthen the bond between our two nations, promoting academic and cultural exchange while standing together in the face of adversity.

We believe that together we can build a brighter future founded on cooperation.

Programme summary

DAY 1
FRIDAY, 16 MAY

12:00 -	**REGISTRATION**
15:00 - 15:15	**OPENING**
15:15 - 16:05	**PLENARY LECTURE** MARIA BARBOSA
16:05 - 17:20	**DISCUSSION PANEL**
17:20 - 17:30	**COFFEE BREAK**
17:30 - 18:30	**ORAL SESSIONS**
18:30 - 18:40	**COFFEE BREAK**
18:40 - 19:30	**PLENARY LECTURE** LARYSA BARABAN
19:30	**OUTDOOR GAME**

DAY 2
SATURDAY, 17 MAY

08:30 - 10:30	**WORKSHOPS**
10:30 - 11:20	**PLENARY LECTURE** CECILIA LANNY WINATA
11:20 - 11:40	**COFFEE BREAK**
11:40 - 12:40	**ORAL SESSIONS**
12:40 - 13:00	**COFFEE BREAK**
13:00 - 13:50	**PLENARY LECTURE** KASPER KARLSSON
13:50 - 15:40	**LUNCH**
15:40 - 16:40	**ORAL SESSIONS**
16:40 - 18:10	**POSTER SESSION & COFFEE BREAK**
18:10 - 19:00	**PLENARY LECTURE** LEONIE LUGINBUEHL
19:00	**SOCIAL EVENT**

DAY 3
SUNDAY, 18 MAY

10:00 - 11:00	**ORAL SESSIONS**
11:00 - 11:50	**PLENARY LECTURE** TOMASZ WŁODARSKI
11:50 - 13:20	**POSTER SESSION & COFFEE BREAK**
13:20 - 14:00	**BRUNCH**
14:00 - 15:15	**DISCUSSION PANEL**
15:15	**CLOSING & PRIZES**

Agenda

Friday, 16 May 2025

12:00 – **Registration**

15:00 – 15:15 **Opening Ceremony** (Room 107)

15:15 – 16:05 **Plenary Lecture** (Room 107)

PL-1 Hypes, Hopes, and the way forward for Microalgal Biotechnology

Maria Barbosa, *Wageningen University and Research Center (NL)*

16:05 – 17:20 **Discussion Panel** (Room 107)

D-1 The impact of scientists on science and society in turbulent times

Panelists: *Kasper Karlsson, Larysa Baraban, Marcin Szymon Filipiak, Rafał Derlacz*

17:20 – 17:30 **Coffee Break**

17:30 - 18:30 **Parallel oral session: "Size Matters: Nano Edition"** (Room 107)

O-1 Bimetallic nanozymes as labels for a novel approach to signal generation in paper-based immunoassays

Paweł Stańczak, *Warsaw University of Technology (PL)*

O-2 Hybrid Magnetic-Core-Based Nanozymes: A Versatile Platform for Bioanalytical and Biomedical Applications

Jan Górniaszek, *Warsaw University of Technology (PL)*

O-3 Theranostic properties of oxygen nanobubbles

Bartosz Płóciennik, *Jagiellonian University, Kraków (PL)*

O-4 Sponge-like titania nanotubes with M13 bacteriophages for *Escherichia coli* detection

Anna Karbarz, *Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw (PL)*

17:30 – 18:30 **Parallel oral session: "Smells like gene spirit"** (Room 329)

O-5 Illuminating the Unseen: Microbial Rhodopsins in Freshwater Lakes

Małgorzata Malczewska, *University of Warsaw (PL)*

O-6 A bone to pick with ancient DNA! Evaluating auditory ossicles as a new resource for genetic studies.

Kajetan Lubacki, *Adam Mickiewicz University, Poznań (PL)*

O-7 High-Throughput Analysis of Chloroplast tRNA Modifications and Their Changes Under High Light Stress

Lidia Muszyńska, *Warsaw University of Life Sciences (PL)*

O-8 Unraveling the role of eriophyoid mites in the spread of raspberry leaf blotch emaravirus (RLBV). Implications for virus transmission and control

Natalia Jeznach, *Warsaw University of Life Science (PL)*

18:30 – 18:40 **Coffee Break**

18:40 – 19:30 **Plenary lecture** (Room 107)

PL-2 Nanoelectronics for cancer immunotherapy

Larysa Baraban, *Helmholz-Zentrum Dresden & Technische Universität Dresden (DE)*

19:30 – 20:30 **Outdoor Game** (Pole Mokotowskie)

Saturday, 17 May 2025

08:30 – 10:30 **Workshops** (Rooms 3xx)

W-1 On the production line — you decide

Małgorzata Ulanowicz&Emilia Cywińska, *Bioton (PL)*

W-2 Design thinking in biotechnology

Wiktoria Frączek, *Warsaw University of Life Sciences (PL)*

W-3 Bioinformatics essentials: Programming the flow of genetic information

Michał Stanowski, *University of Warsaw (PL)*

W-4 Basics of bioprinting with BioCloner Health

Julia Talecka&Jakub Knap-Wardzyński, *BioCloner Health (PL)*

10:30 - 11:20 **Plenary lecture** (Room 107)

PL-3 Decoding Heart Development and Disease Through Zebrafish Genomics

Cecilia Winata, *International Institute of Molecular and Cell Biology, Warsaw (PL)*

11:20 – 11:40 **Coffee Break**

11:40 – 12:40 **Parallel oral session: "The Dermatrix"** (Room 107)

O-9 Upcycling spent coffee grounds: a natural source of antioxidants and oils for cosmetics

Zoja Trojan, *Warsaw University of Technology (PL)*

O-10 Semi-synthetic ceramides influence on skin cell viability and cellular behavior in 2-dimensional (2D) and 3-dimensional (3D) models

Iryna Levkovych, *Warsaw University of Technology (PL)*

O-11 Spent coffee grounds as a source of antioxidant extract with caffeine and phenolic acids with potential effects on skin cells

Adrianna Piasek, *Warsaw University of Technology & EcoBean sp. z o.o., Warsaw (PL)*

O-12 Unveiling the Secrets of Melanogenesis: Depigmenting Agents in Melanoma

Julia Sępioł, *Warsaw University of Technology (PL)*

11:40 – 12:40 **Parallel oral session: "Molecular Panic"** (Room 329)

O-13 BrewCure — From Waste to Wellness: Unveiling the Biological Potential of Arabica and Robusta Coffee Waste Extracts

Karolina Mikulska, *Warsaw University of Technology & Scientific Association HERBION, Warsaw (PL)*

O-14 The impact of pollution on the electrophysiology of epithelium — observations from Caco-2 cell model

Gabriela Węglińska, *Warsaw University of Life Sciences (PL)*

O-15 Heme oxygenase-1 affects nuclear envelope structure

Eryk Chatian, *Jagiellonian University, Kraków (PL)*

O-16 GYY4137, a slow-releasing hydrogen sulfide donor attenuates fibrosis and inflammation in the diaphragm of dystrophic D2.*mdx* mice

Anna Nalepa, *Jagiellonian University, Krakow (PL)*

12:40 – 13:00 **Coffee Break**

13:00 – 13:50 **Plenary lecture** (Room 107)

PL-4 Winding back the tape of tumors

Kasper Karlsson, *Karolinska Institutet, Stockholm (SE)*

13:50 – 15:40 **Lunch** (Central Canteen)

15:40 – 16:40 **Parallel oral session: "Strain Wars"** (Room 107)

O-17 Sub-mic antibiotic exposure promotes hetero-resistance in bacteria

Shakeel Ahmad, *Institute of Physical Chemistry Polish Academy of Sciences, Warsaw (PL)*

O-18 Relation between body mass and vaginal microbiome of patients with polycystic ovarian syndrome diagnosis

Maria Bocheńska, *Adam Mickiewicz University, Poznań (PL)*

O-19 4 types of healthy uterus microbiome

Katarzyna Morańska, *Adam Mickiewicz University, Poznań (PL)*

O-20 Substrate geometry affects population dynamics in a bacterial biofilm

Klaudia Staśkiewicz, *Institute of Physical Chemistry PAS, Dioscuri Centre for Physics and Chemistry of Bacteria, Warsaw (PL)*

15:40 – 16:40 **Parallel oral session: "Bloodbusters"** (Room 329)

O-21 Regulation of Hematopoietic Stem Cell Differentiation by the Neogenin-1/Netrin-1 Axis

Mateusz Sar, *Jagiellonian University, Kraków (PL)*

O-22 Stroma-driven horizontal transfer of TCA proteins enhances metabolic plasticity and promotes imatinib resistance in chronic myeloid leukemia

Nikodem Kasak, *Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw (PL)*

O-23 SF1 is translationally controlled through conserved 5' UTR to regulate differentiation of blood

Daniel Grygorowicz, *International Institute of Molecular Mechanisms and Machines, Polish Academy of Sciences, Warsaw (PL)*

O-24 The importance of platelet growth factors as markers of survival and severity of COVID-19 in patients undergoing monthly follow-up

Urszula Łacek, *Pomeranian Medical University, Szczecin (PL)*

16:40 – 18:10 **Poster session 1: P-1–P-49**

18:10 – 19:00 **Plenary lecture** (Room 107)

PL-5 Understanding and optimizing the cost–benefit balance of plant–fungal symbioses

Leonie Luginbuehl, *University of Cambridge (UK)*

19:00 – 21.00 **Social Event** (MiNI)

21:00 – **Conference Party** (Pardon, To Tu)

Sunday, 18 May 2025

10:00 – 11:00 **Parallel oral session: "Trust me, I am an engineer"** (Room 107)

O-25 Development of an Organ-on-a-Chip Microsystem for Endometrium Modeling

Agnieszka Jankowska, *Warsaw University of Technology (PL)*

O-26 Design and Construction of a Magnetic Microreactor for Enzymatic Reactions

Kacper Dybizbański, *West Pomeranian University of Technology, Szczecin (PL)*

O-27 Research on the use of polymer membranes with magnetic properties for the culturing and maturation of heart cells

Katarzyna Linek, *Warsaw University of Technology (PL)*

O-28 Statins Reloaded: Investigating the Oncological Potential of Cardiovascular Drugs

Magdalena Twardowska, *Rzeszow University of Technology (PL)*

10:00 – 11:00 **Parallel oral session: "Think outside of the cell"** (Room 329)

O-29 Anisakis simplex extracellular vesicles as modulators of oxidative stress in host cells

Magdalena Stawicka, *University of Warmia and Mazury, Olsztyn (PL)*

O-30 The impact of *Toxocara canis* antigens on the cytokine response of lung epithelial cells and macrophages in an *in vitro* co-culture model

Monika Woźniak, *Warsaw University of Life Sciences (PL)*

O-31 Analysis of the expression and activity of proteolytic enzymes of the pitcher plant (Nepenthes x ventrata) during nitric oxide-supplemented digestion

Jakub Bieniek, *Warsaw University of Life Sciences (PL)*

O-32 Apis mellifera royal jelly-derived extracellular vesicles as a potential anti-aging tool.

Mikołaj Panek, *Wrocław University of Environmental and Life Sciences (PL)*

11:00 – 11:50 **Plenary lecture** (Room 107)

PL-6 Unravelling protein folding in cells through a computational microscope

Tomasz Włodarski, *Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw (PL)*

11:50 – 13:20 **Poster session 2 (P-50–P-95)**

13:20 – 14:00 **Brunch** (MiNI)

14:00 - 15:15 **Discussion panel** (Room 107)

D-2 Future is now — support and development of Polish biotechnology

Panelists: *Gajane Żurawska, Maciej Gołaszewski, Aleksandra Izdebska*

15:15 – **Closing & Prizes** (Room 107)

Poster Session 1

Saturday, 17 May, 16:40 – 18:10

P-1 Proteolytic processing of adhesion GPCR

Marta Kowalska, *Jagiellonian University, Kraków (PL)*

P-2 The potential role of arachidonic acid derivatives as biomarkers for assessing prognosis and mortality in COVID-19 patients during a month observation

Adrianna Jerzyk, *Pomeranian Medical University in Szczecin (PL)*

P-3 The Role of Nuclear Factors in Programmed Cell Death Induction in CRISPR/Cas9-Modified Cells

Jakub Pawlikowski, *Silesian University of Technology, Gliwice (PL)*

P-4 Optimizing whole-grain bread recipe with microencapsulated polyphenols for functional benefits

Weronika Bińkowska, *Warsaw University of Life Sciences (PL)*

P-5 The antioxidant and anti-aging activity of Helichrysum arenarium callus tissue

Joanna Jabłońska, *Medical University of Warsaw (PL)*

P-6 Effect of magnetic fields on L929 murine fibroblasts

Marta Maślanko, *West Pomeranian University of Technology in Szczecin (PL)*

P-7 The influence of selected methylxanthines on the interactions with pulmonary surfactants at the water-air interface

Wiktoria Kołomyjska, *University of Warsaw (PL)*

P-8 On the role of intercalating residues in restriction associated dsDNA endonucleases

Norbert Osiński, *Jagiellonian University, Kraków (PL)*

P-9 Characteristics of oils extracted from sunflower seeds roasted with thyme

Tymoteusz Kołodziejczyk, *Warsaw University of Life Sciences (PL)*

P-10 Novel droplet-based microcapsule system for microbial growth.

Karol Wojciechowski, *University of Warsaw (PL)*

P-11 Investigating the oligomerization of IFIT1 protein involved in the cellular immune response

Joanna Grzymkowska, *Warsaw University of Technology (PL)*

P-12 Natural products as bioinsecticides — effects of solamargine on the immune-related mechanisms in *Tenebrio molitor* L.

Natalia Bylewska, *Adam Mickiewicz University in Poznań (PL)*

P-13 *Porphyromonas gingivalis* and *Bacteroides fragilis* HmuS — a new family of proteins with dechelatase activity

Patryk Cierpisz, *University of Wrocław (PL)*

P-14 Green nanoparticle synthesis in the application of non-bacterial mastitis in cattle

Michał Motrenko, *Warsaw University of Life Sciences (PL)*

P-15 Advancing 3D Printing in Dentistry: Study on Printing Accuracy and Photopolymerization Shrinkage

Jakub Pietraszewski, *Cracow University of Technology (PL)*

P-16 Antibacterial and anticancer properties of kombucha enriched with onion juice or dandelion root juice

Barbara Utrata, *Warsaw University of Life Sciences (PL)*

P-17 Smart sensors for smarter healing: aptamer layers and printed electronics in wound care

Julia Czopińska, *Warsaw University of Technology (PL)*

P-18 Optimization of Biomass Production Parameters for Probiotic Microorganisms *Lactobacillus apis* and Bombella apis

Alina Sobolieva, *Lviv National Stepan Gzhytsky University of Veterinary Medicine and Biotechnologies (UA)*

P-19 Exploring Hormetic Effects: Sulforaphane's Role in Triple-Negative Breast Cancer via In Vitro and In Vivo Models

Anna Pogorzelska, *National Medicines Institute, Warsaw (PL)*

P-20 Selenium nanoparticles obtained by the green and chemical synthesis as antibacterial agents

Jakub Szmytke, *University of Warsaw (PL)*

P-21 Effects of transient oxygen and glucose deficiency on rat oligodendroglial mitochondria in in vitro model of neonatal hypoxia–ischemia

Michał Frańczak, *Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw (PL)*

P-22 Toxic potential of *Bacillus cereus* sensu lato strains isolated from food

Milena Maliszewska, *University of Białystok (PL)*

P-23 Migration and Invasion Properties of Glioblastoma Cells in Hypoxia

Julia Kozik, *Jagiellonian University, Kraków (PL)*

P-24 The effect of cytokines on the antioxidant properties of Polyscias filicifolia (C. Moore ex E. Fourn.) L. H. Bailey (Araliaceae) callus extracts

Joanna Szatko, *Warsaw Medical University (PL)*

P-25 Silencing Id1 in glioma-associated microglia using targeted nanocarriers to restore their antitumor function

Paulina Dulanowska, *University of Warsaw (PL)*

P-26 Antibacterial potential of biosurfactants produced by *Bacillus subtilis* WA51 and their interactions with plant compounds

Inga Suchodolska, *University of Warsaw (PL)*

P-27 Identifying molecular determinants of bacterial adhesion to biotic and abiotic surfaces in *Ochrobactrum* spp.

Aleksandra Karpińska, *University of Gdańsk & Medical University of Gdansk (PL)*

P-28 Visualisation of hypoxia-induced changes in cellular NAD+ levels

Kinga Serafin, *Jagiellonian University, Kraków (PL)*

P-29 In vitro evaluation of wild strawberry leaf and acerola fruit extracts: effects of NaOH and AgNPs enrichment on macrophage-mediated inflammation

Kinga Suska, *Medical University of Lodz (PL)*

P-30 Microplatform for vascularization of three-dimensional cardiac cell cultures

Aleksandra Posłuszny, *Warsaw University of Technology (PL)*

P-31 Bacterial cellulose as an alternative protectant in the microorganism storage process.

Nikola Kabała, *West Pomeranian University of Technology, Szczecin (PL)*

P-32 Studies on the mitochondrial genome of holoparasitic plants from Orobanchaceae family

Anna Burda-Uryga, *Jagiellonian University, Kraków (PL)*

P-33 Development of a highly stable and soluble variant of FGF8 for biomedical applications

Kamila Skrzyńska, *University of Wroclaw, (PL)*

P-34 Exploring the pleiotropic effects of isoxazole derivative of usnic acid in breast cancer cells

Klaudia Żuczek, *University of Gdańsk (PL)*

P-35 Same Microbial Oil, Different Outcome? The Impact of Extraction Methods on Composition of oleaginous yeast lipids.

Paulina Goleń, *Warsaw University of Life Sciences (PL)*

P-36 The influence of chemotherapeutic stress and hypoxia on the metabolic plasticity of human glioblastoma multiforme

Aldona Szewczyk, *Jagiellonian University, Krakow (PL)*

P-37 Investigation of the transient increase in blood-brain barrier permeability using a microsystem.

Weronika Pietrzyk, *Warsaw University of Technology (PL)*

P-38 Biosensing albumin with new benzylidene derivatives — investigation of fluorophore–protein interactions

Małgorzata Kowalewska, *Cracow University of Technology (PL)*

P-39 Elevated glucose levels reduce the degradation of amyloid precursor protein (APP)

Zuzanna Terlikowska, *University of Gdansk (PL)*

P-40 Microbial hitchhikers: How pollinators shape nectar microbiomes across plant species

Bartłomiej Starzyński, *University of Warsaw (PL)*

P-41 Studying the role of LINC00116 in lymphomas: Does it function as a long non-coding RNA?

Paulina Mędrala, *Silesian University of Technology, Gliwice (PL)*

P-42 TRIM21 as a molecular link between inflammation and metabolic dysregulation in diabetes

Anna Wojtas, *Jagiellonian University, Kraków (PL)*

P-43 The Importance of AlkB Deoxygenate and AidB Dehydrogenase in the Repair of Damaged RNA Bases

Dagmara Koperska, *Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw (PL)*

P-44 Innovative Iridium(III) Complexes as Theranostic Photosensitizers for Photodynamic Therapy (PDT) and Sensors for High-Precision Single-Cell Imaging.

Weronika Wielgus, *Cracow University of Technology (PL)*

P-45 *Sarracenia purpurea* L.: from carnivorous to medicinal

Kinga Maria Pilarska-Dudziak, *Wrocław University of Environmental and Life Sciences (PL)*

P-46 Analysis of specific metabolites in selected carnivorous plants grown under in vivo conditions

Mateusz Lipiński, *Wrocław University of Environmental and Life Sciences (PL)*

P-47 Comparative analysis of the efficiency of photothermal therapy using gold nanostars and gold-coated iron oxide nanoparticles

Julia Husiatyńska, *Warsaw University of Technology (PL)*

P-48 The role of Heme Oxygenase 1 in stress granule formation. Reevaluation of Integrated Stress Response

Jan Pacześniak, *Jagiellonian University, Kraków (PL)*

P-49 The role of glutathione in CRISPR-Cas9-modified cells

Maciej Ejfler, *Silesian University of Technology, Gliwice (PL)*

Poster Session 2

Sunday, 18 May, 11:50 – 13:20

P-50 Microbial consortium-driven strategies for enhanced sewage sludge composting and environmental impact mitigation

Julia Rydz, *University of Warsaw (PL)*

P-51 Gene expression profile for cysteine cathepsins and resulting TLR-IRF pathways in murine cDC subsets

Adrianna Niedzielska, *Warsaw University of Life Sciences (PL)*

P-52 Purification and characterization of endocrine fibroblast growth factors (FGFs): the example of FGF19

Szymon Bander, *University of Wroclaw (PL)*

P-53 Biotechnological approach to *Aralia cachemirica* Decne: transformation and in vitro cultivation of hairy roots for biodiversity conservation

Marta Bedra, *Medical University of Warsaw (PL)*

P-54 Development of a novel microfluidic chip for thermotaxis-based sperm selection

Filip Kozłowski, *Warsaw University of Technology (PL)*

P-55 Panel design for the study of macrophage activity during the course of sepsis

Aleksander Lempert, *Medical Centre for Postgraduate Education, Warsaw (PL)*

P-56 DXO1 involved in biotic stress response

Wiktoria Kalbarczyk, *University of Warsaw (PL)*

P-57 Characterization of Lipid Composition in *Yarrowia lipolytica* Microbial Oil Derived from Alternative Carbon Sources

Aleksandra Piotrowicz, *Warsaw University of Life Sciences (PL)*

P-58 Refining the role of RIG-I in dsRNA recognition: the impact of 5′ end and epitranscriptomic modifications

Wiktoria Szymanek, *University of Warsaw (PL)*

P-59 Bone marrow-derived mouse neutrophils as a model for NETs in biomedical studies

Pola Pruchniak, *Warsaw University of Life Sciences (PL)*

P-60 Application of Morphologically Chiral Gold Nanorods in Plasmonic ELISA for TNFα Detection

Malwina Hamera, *University of Warsaw (PL)*

P-61 Role of clathrin- and caveolin-dependent endocytosis in alphaherpesvirus (EHV-1, HHV-1) entry into murine neuron cells — in vitro studies

Maria Kalenik, *Warsaw University of Life Sciences (PL)*

P-62 Regulatory Role of the sRNA OmrA in the High-Pathogenicity Island of *Yersinia enterocolitica*

Paulina Lipska, *University of Warsaw (PL)*

P-63 Construction and phenotypic analysis of mutants in pili synthesis-related genes in *Pseudomonas donghuensis* strain P482.

Mikołaj Pęczak, *University of Gdansk & Medical University of Gdansk (PL)*

P-64 The influence of elicitation on antioxidant properties of *Polyscias filicifolia* (C. Moore ex E. Fourn.) L. H. Bailey (Araliaceae) callus extracts

Julianna Warchoł, *Medical University of Warsaw (PL)*

P-65 What's new with *Kluyveromyces marxianus*? Exploring its therapeutic potencial

Marta Rogalska, *Warsaw University of Technology (PL)*

P-66 Plant tRNA modifying enzyme MIAA influences chloroplasts translation, photosynthesis and cold stress responses

Martyna Adach, *Warsaw University of Life Sciences (PL)*

P-67 Construction of a Titanium-Binding T7 Bacteriophage Using the Phage Display Method

Aleksandra Głowacka, *University of Warsaw (PL)*

P-68 Assessing the potential benefits of humic substances extracted from sewage sludge

Justyna Michalska, *Silesian University of Technology, Gliwice (PL)*

P-69 In search of Cytokinin Homeostasis: The Interplay of CKX2 and PUP7 in Rye

Justyna Jazowska, *Plant Breeding and Acclimatization Institute — National Research Institute, Błonie (PL)*

P-70 Barcoding Droplet Composition in Droplet Screen-seq: A New Platform for Ultra-High Throughput Microbial Consortia Analysis

Józef Krzak, *University of Warsaw (PL)*

P-71 γ-decalactone as an active compund in edible packaging films

Anna Pakulska, *Warsaw University of Life Sciences (PL)*

P-72 Development and characterization of a highly stable FGF2 variants and their role in cell signaling, migration and glucose uptake

Szymon Sidor, *University of Wroclaw (PL)*

P-73 Cell line-specific metabolic adaptations in glioblastoma: LDH as a marker in 2D and 3D cultures

Dominika Blicharska, *Jagiellonian University, Kraków (PL)*

P-74 Identifying new effector genes of the mitochondrial retrograde pathway in *Candida albicans*

Marta Dilling, *University of Warsaw (PL)*

P-75 AI-supported image analysis of droplet deformation for high-throughput and label-free measurement of microbial proteolytic activity

Maciej Andrzejewski, *University of Warsaw (PL)*

P-76 Identification of a pathogenic variant of COL1A1 in a 10-years-old donor diagnosed with OI type I

Malwina Botor, *Medical University of Silesia, Katowice (PL)*

P-77 Bismuth(III) complexes of 1,2,4,5-tetrasubstituted imidazoles as promising antimicrobial and urease-inhibitory agents

Viktoryia Staravoitava, *Jagiellonian University, Kraków (PL)*

P-78 Analysis of prokaryotic symbionts among eglenozoan protists

Julia Kawa, *Uniwersytet Warszawski (PL)*

P-79 Transcriptomic and physiological responses of Umbelopsis to endohyphal bacteria — Paraburkholderia

Maria Furman, *University of Warsaw (PL)*

P-80 Fed-Batch Cultivation of Yarrowia lipolytica: Lipid Production and Supernatant Valorization

Suheda Ugur, *Warsaw University of Life Sciences (PL)*

P-81 Unveiling the secrets of stroma-leukemia communication and its pro-leukemic outcomes: focus on the CD44 protein

Laura Turos-Korgul, *Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw (PL)*

P-82 Rickettsia diversity and prevalence in *Dermacentor reticulatus* ticks from two expansion zones in Poland

Patrycja Puszko, *University of Warsaw (PL)*

P-83 ChatGPT in Higher Education — A Learning Aid or an Obstacle?

Igor Ramenskii, *University of Gdańsk (PL)*

P-84 Synthesis of the m7GpppG-AuNP Conjugate and Investigation of Its Ability to Bind eIF4E Protein Using Gel Electrophoresis

Agata Wagner, *University of Warsaw (PL)*

P-85 No lysines, no problem? How FBXL15 breaks the rules of ubiquitination

Gabriela Piórkowska, *International Institute of Molecular and Cell Biology, Warsaw (PL)*

P-86 Synthesis and biological evaluation of TBBi derivatives as potential CK2 inhibitors with anticancer properties

Egor Fedorov, *Warsaw University of Technology (PL)*

P-87 Study of WHIRLY1 protein turnover in *Arabidopsis thaliana*

Adrianna Sip, *Adam Mickiewicz University in Poznań (PL)*

P-88 Impact of T cell activation on lysosomal function during inflammation

Martyna Kuczyńska, *University of Gdansk (PL)*

P-89 Study of the influence of selected substrate parameters on the SERS effect

Joanna Koprowska, *Institute of Physical Chemistry Polish Academy of Sciences, Warsaw (PL)*

P-90 Development of bacterial cellulose-PLA composites via 3D printing and in situ biosynthesis

Adam Truszczyński, *West Pomeranian University of Technology in Szczecin (PL)*

P-91 Phenytoin reduces hypersensitivity by altering microglia/macrophage activity at the spinal cord level in a rat model of neuropathic pain

Anna Kusiak, *Maj Institute of Pharmacology Polish Academy of Sciences, Kraków (PL)*

P-92 The influence of selected material properties of bacterial cellulose membranes on the permeability of inorganic salt compounds.

Weronika Runowska, *West Pomeranian University of Technology in Szczecin (PL)*

P-93 In silico expression of human protein DYRK1A in *Pseudoalteromonas haloplanktis* 125

Joanna Krajewska, *University of Warsaw (PL)*

P-94 Development of a new Rabies virus with changed tropism

Lucyna Piórkowska, *International Centre for Translational Eye Research, Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw (PL)*

P-95 Development of a novel microfluidic approach for identifying and tracking horizontal gene transfer events

Kornelia Biegańska, *University of Warsaw (PL)*

PL-1: Hypes, Hopes, and the way forward for Microalgal Biotechnology

Maria Barbosa[*,1]

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The urge for food security and sustainability has advanced the field of microalgal biotechnology. Microalgae are microorganisms able to grow using (sun)light, fertilizers, sugars, CO_2, and seawater. They have high potential as a feedstock for food, feed, energy, and chemicals. Microalgae grow faster and have higher areal productivity than plant crops, without competing for agricultural land, and with 100% efficiency uptake of fertilizers. In comparison with bacterial, fungal, and yeast single-cell protein production, based on hydrogen or sugar, microalgae show higher land use efficiency. New insights are given into the potential of microalgae replacing soy protein, fish oil, and palm oil, and being used as cell factories in modern industrial biotechnology to produce designer feed, recombinant proteins, biopharmaceuticals, and vaccines.

Prof. dr Maria Barbosa is Professor in Bioprocess Engineering and is Director of AlgaePARC at Wageningen University and Research Centre, the Netherlands. She has been president of the Dutch Biotechnology Association (NBV) and is a member of the European Algae Biomass Association (EABA) steering group. She holds a Ph.D. in Bioprocess Engineering obtained at Wageningen University. She has worked at ETH (Swiss Federal Institute of Technology), Switzerland, IBET, and at EMBO (European Molecular Biology Organisation), Germany. She presently leads the group on microalgal biotechnology and coordinates several large research programs covering the entire microalgae production chain. Her scientific interests are in microalgae strain improvement, process design and scale-up.

PL-2: Nanoelectronics for cancer immunotherapy

Larysa Baraban[*,1]

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Cancer is an extraordinarily diverse disease that affects our society globally; it hits people of all ages and genders. Because of its high mortality rate, it is responsible on average for one in six deaths, and it is the second most common cause of death, according to the World Health Organization [1]. During the last decade, new ways of stimulating the inherent ability of our immune system to efficiently attack tumors were found, which was distinguished by the Nobel Prize in Physiology and Medicine 2018 for James P. Allison and Tasuku Honjo. Currently, entirely new principles for cancer immunotherapy are on the rise, based on the inhibition of immune checkpoints on the surface of T-cells (e.g. PD-1, CTLA-4, LAG-3, etc.) or respective ligands on the cancer cells (e.g. PD-L1); and cross-linking of immune cells to target cancer cells by e.g. bispecific antibodies or engineered CAR T-cells.

Still, the complexity of every individual cancer limits the efficacy of immunotherapy to a maximum of 20-40% of clinical patients [2]. Even if cancer genetics is correctly determined, complex and unique cancer ecosystems may prevent successful immunosurveillance. As the so-called cancer-immunity cycle – a set of processes the immune system does to eliminate cancer – is damaged in cancer patients, the task of immunotherapy is to repair the breaches in the immunity cycle, via 'external' support, either with mono- or combinational treatment. Thus, the main goal in the community is to achieve transformative treatment results for more patients, via better immune cancer phenotyping [3]. Nanosensor devices merged with biological species, e.g. cells or molecules of similar nanosize, offers a remarkable increase in biosensor sensitivity. Despite this success, nanobioelectronics is still underrepresented in the field of oncology. While the research area has addressed their potential applications in early cancer diagnosis less efforts have been dedicated to the therapy development and patients monitoring. In treatment monitoring, potential use cases are mostly limited by liquid biopsy, detection of circulating tumor cells or circulating tumor DNA. The recent works extend the borders of the silicon based nanosensor applications to the field of cancer immunology, i.e. contributing to the optimization of the novel CAR T cell immunotherapy for the prostate stem cell antigens as well as against fibroblasts activation proteins [4]. Here silicon nanowire field-effect transistors are used to pre-select targeting molecules for an adaptive CAR-T cell operation. Focusing on a library of seven variants of E5B9 peptide that is used as CAR targeting epitope, we performed multiplexed binding tests using nanosensor chips. The correlation of binding affinities and sensor sensitivities enabled a selection of candidates for the interaction between CAR and target modules. Furthermore, we demonstrate the ability to monitor the immunotherapeutic drugs in vivo, using the electronic platform.

In conclusions, the research landscape in the last few years has shown encouraging signs for nanoelectronics to be better represented in cancer research in near future. In particular, this approach can open new routes to perform a complex combinatorial analysis using tiny electronic chips and simultaneously screening multiple biochemical species thus facilitating the transition from conventional medicine to precision medicine in clinical oncology.

Research is supported by the European Research Council (ERC) in frames of a Consolidator Grant (ImmunoChip, 101045415)

[1] World Health Organization. (2022) “Cancer – fact sheet” (website).
[2] Sharma P. et al. (2017) Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell 168: 707-723.
[3] Chen D., Mellman I. (2013) Oncology Meets Immunology: The Cancer-Immunity Cycle. Immunity 39: 1-10.
[3] Nguyen-Le T. et al. (2022) Nanosensors in clinical development of CAR-T cell immunotherapy. Biosensors and Bioelectronics 206: 114124.
[4] Nguyen-Le T. et al. (2025) Toward Personalized Immunotherapeutic Drug Monitoring with Multiplexed Extended-Gate Field-Effect-Transistor Biosensors. Small Science 5: 2400515.

Larysa Baraban is a Professor at the Faculty of Medicine Carl Gustav Carus, Dresden University of Technology, which is a joint professorship through the Initiative and Networking Fund of Helmholtz Association. She is also the Head of ‘Nano-microsystems for life sciences’ Department at the Institute of Radiopharmaceutical Cancer Research, Helmholtz Center Dresden Rossendorf. Prof. Baraban received her PhD in Physics from the University of Konstanz in 2008. Between 2013 and 2019 she led the “BioNanoSensorics” group within the Chair of Materials Science and Nanotechnology at the Institute for Materials Science and Max Bergmann Centre for Biomaterials, Faculty of Mechanical Science and Engineering, Technische Universität Dresden. In 2022, she received a prestigious ERC Consolidator Grant for the project ‘ImmunoChip’: Nano-assisted digitalizing of cancer phenotyping for immunotherapy (project number: 101045415).

PL-3: Decoding Heart Development and Disease Through Zebrafish Genomics

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The heart performs a vital function in circulating oxygen-rich blood and nutrients throughout the body. The core genetic program underlying heart development is largely conserved across metazoans, and aberrations to this process can result in congenital heart disease. While key transcription factors such as Gata5, Tbx5a, Hand2 are known to regulate cardiogenesis, their downstream gene networks and chromatin-level mechanisms remain unresolved. Using the zebrafish model organism, we integrate transcriptomics and epigenomics (ATAC-seq) to characterize the transcriptome and chromatin landscape during heart development. Our analyses revealed dynamic gene expression and chromatin rearrangements throughout different stages of heart morphogenesis, likely representing genetic regulatory hubs driving key events of heart development. Furthermore, the loss of cardiac transcription factors perturbs global chromatin accessibility, particularly at evolutionarily conserved non-coding regions, suggesting these as candidate enhancers involved in heart development. To elucidate the role of these enhancers in heart development and disease, we employ both experimental and computational approaches for discovery and functional validation in the zebrafish. Ultimately, we aim to define the contribution of the dynamic transcriptional regulatory landscape to heart development and identify novel elements (both genic and non-genic) associated with congenital heart disease.

Dr. hab. Cecilia Winata completed her PhD in Biology at the National University of Singapore. She then joined the Genome Institute of Singapore as a postdoctoral fellow. During this time, she worked on several projects which pioneered the use of next generation sequencing (NGS) to study zebrafish gene regulation. In 2014, she became a Max Planck/IIMCB group leader at the International Institute of Molecular and Cell Biology in Warsaw, Poland, where she established zebrafish as a research model at the institute. Her lab integrates genomics, epigenetics, and experimental embryology to study transcriptional regulation in heart development, post-transcriptional control of maternal mRNAs, and rare genetic diseases using zebrafish models. By combining CRISPR-Cas9, single-cell omics, and clinical collaborations, her team bridges fundamental developmental insights with human disease mechanisms.

PL-4: Winding back the tape of tumors

Kasper Karlsson[*,1]

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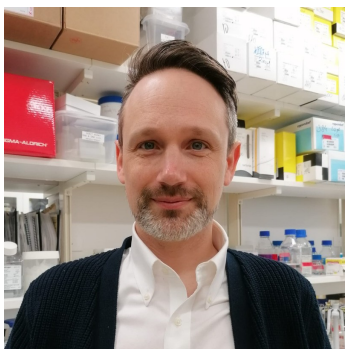
Stephen J. Gould once famously asked: "Should the tape of life be replayed, would it produce similar living beings?". This question may never get fully resolved, but recent technological developments allowed us to ask a similar question transposed to the context of cancer: "If we initiate tumor evolution in multiple similar cells, will the same phenotype emerge?". To tackle this problem, we made use of organoid cell culture models that recapitulates native tumors with increasing fidelity, and expressed cellular barcodes that make it possible to study evolutionary processes with high resolution, to establish an experimental ex vivo platform to study tumorigenesis. Specifically, TP53-deficiency was engineered into primary human gastric organoids from healthy donors and several clonally derived replicate cultures were evolved for over two years. The cultures were extensively interrogated by DNA and single cell RNA sequencing, and cell barcoding was introduced to examine subclonal dynamics. We observed that several features of gastric tumor evolution could be replicated by prolonged ex vivo culturing, that selection was similar across replicates from the same culture and that cultures from different donors evolved similar malignant phenotypes. This suggests that tumor evolution is partly intrinsic to the tumor cells, without the influence of a complex microenvironment, and moreover that this process to some extent is predictable. By "restarting the tape of cancer" under different conditions, we may find a path forward that thwart malignant transformation.



Dr. Kasper Karlsson started his research career and PhD in 2011 at Karolinska Institutet in Stockholm, Sweden, where he was part of the team that developed the unique molecular identifier strategy, now widely used in single cell sequencing experiments, to correct for amplification induced bias. In 2016 he moved to Stanford, USA, for his postdoctoral studies, where he developed in vitro model systems of tumor evolution. Using organoid technology, he showed that some aspects of early gastric tumor evolution can be recapitulated in cell culture models. Kasper is an assistant professor at Karolinska Institutet, where he develops new therapies and drug combinations for pediatric cancer, including the concept "Precision Lethality" where cell barcoding is used to identify and target cell populations that are resistant to standard of care with new drugs.

PL-5: Understanding and optimizing the cost-benefit balance of plant-fungal symbioses

Leonie Luginbuehl[*,1]

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The arbuscular mycorrhizal (AM) symbiosis between plants and beneficial soil fungi provides key benefits to plants: AM fungi help the host plant to take up essential mineral nutrients and water from the soil, thereby increasing crop yield by up to 30%. However, the symbiosis comes at a cost. In return for soil nutrients, plants allocate up to 20% of their photosynthetically assimilated carbon to the fungal partner. This symbiotic carbon transfer has a major impact not just on plant and fungal physiology, but also on the global carbon cycle. In this talk, I will provide an overview of our current knowledge on how assimilated carbon is transferred from plants to AM fungi. I will also present our current work, which aims to understand how carbon allocation to AM fungi is regulated by the plant and how we might be able to exploit these regulatory mechanisms to maximise the benefits of the mycorrhizal symbiosis for sustainable agriculture.

Dr. Leonie Luginbuehl obtained her PhD from the John Innes Centre in Norwich, UK, where she investigated how plant roots are transcriptionally reprogrammed to enable the establishment of a symbiosis with beneficial soil fungi called arbuscular mycorrhizal fungi. Her work identified a lipid biosynthesis and export pathway that provides arbuscular mycorrhizal fungi with fixed carbon in the form of fatty acids. As a Herchel Smith Fellow at the Department of Plant Sciences in Cambridge, Leonie studied the cell-type specific regulation of photosynthesis gene expression in leaves of C3 and C4 plants using single-cell sequencing approaches. In September 2022, she started her own group as Assistant Professor at the University of Cambridge, investigating the regulation of nutrient exchange in the arbuscular mycorrhizal symbiosis. Leonie is also a co-founder of the biotech start-up Crop Diagnostix, a company that uses gene expression biomarkers to diagnose plant stress in crop plants.

PL-6: Unravelling protein folding in cells through a computational microscope

Tomasz Włodarski[*,1]

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Proteins are fascinating biomolecules responsible for virtually every process in our cells. To carry out their functions, most proteins must adopt the correct three-dimensional shape through a process known as protein folding. In cells, this process can begin while the protein is still being synthesised on the ribosome, one of the most ancient and complex molecular machines. Proper folding is critical, as misfolding can lead to severe diseases, including neurodegenerative disorders such as Parkinson's and Alzheimer's.

In our research, we use molecular dynamics simulations ("computational microscope") enriched with experimental data from nuclear magnetic resonance spectroscopy (NMR) and cryogenic electron microscopy (cryo-EM) to provide new insights into cellular protein folding and how the ribosome shapes it. We structurally characterised the folding pathways by capturing the intermediates that emerge in co-translational folding, but are surprisingly absent in vitro.

Thanks to recent advances in cryo-EM, the so-called "resolution revolution", we now have access to structural snapshots of 66 ribosomes from a range of organisms and organelles. By combining these structures with models obtained through breakthroughs in machine learning, exemplified by AlphaFold, we can now start to explore the structural and sequence heterogeneity of ribosomes and their potential influence on protein co-translational folding. Specifically, we have focused on the ribosomal exit tunnel, a long conduit through which the nascent polypeptides reach the outside of the ribosome.

To analyse the geometric features of ribosomal exit tunnels in various structures, we have developed a computational method based on the nascent chain molecular dynamics simulations. With this approach, we characterised the nascent chain pathways in the tunnel and how they change between species and at different stages of biosynthesis. We also performed an extensive bioinformatics analysis of the sequences of proteins forming this ribosome tunnel to comprehensively describe ribosome heterogeneity and investigate the link between the ribosome and the proteome it synthesises.

Dr. Tomek Włodarski is a computational biophysicist and research fellow in the Department of Bioinformatics at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences in Warsaw. He obtained his PhD from the University of Warsaw, applying computational methods to study protein–protein interactions and bioinformatics to identify and characterise novel methyltransferases in the S. cerevisiae genome. During his postdoctoral research, funded by an EMBO Long-Term Fellowship, he worked at University College London in the group of Prof. John Christodoulou, and at the University of Cambridge in the group of Prof. Michele Vendruscolo, developing all-atom and coarse-grained molecular dynamics simulations to study protein folding on the ribosome, guided by experimental restraints from NMR and cryo-EM data. He recently returned to Poland with the POLONEZ BIS grant from the National Science Centre to lead the project "Co-translational protein folding in the light of ribosome evolution", which integrates bioinformatics, multi-scale molecular dynamics simulations, and machine learning to investigate ribosome heterogeneity, particularly at the exit tunnel, and its influence on co-translational protein folding.

D-1: The impact of scientists on science and society in turbulent times

This international panel brings together researchers and biotech industry representatives to reflect on the role of science during recent global crises—including the COVID-19 pandemic, war, and the ongoing climate emergency. The discussion aims to explore how science responded, what challenges it faced, and what lessons can guide us toward building a more resilient, trustworthy, and impactful scientific ecosystem for the future. We will focus on how to improve crisis response, public trust, collaboration, and scientific education—asking what kind of science the world truly needs in times of instability. From our panelists, you can expect critical perspectives on the role of science and biotechnology in addressing global and local challenges, global viewpoints on research collaboration and knowledge transfer and open, honest dialogue with the audience.

The discussion will be moderated by **Bartłomiej Gutkowski**, who will help frame the key questions and facilitate an open exchange of ideas on science in times of crisis.

The panelists

Prof. Larysa Baraban, short bio in plenary lectures section

Kasper Karlsson, short bio in plenary lectures section

Marcin Szymon Filipiak PhD in Chemistry and MBA graduate, currently Assistant Professor at CEZAMAT, Warsaw University of Technology. His work focuses on biosensors for point-of-care diagnostics, with expertise in (bio)electrochemistry, nanomaterials, and microfluidics. He is also a co-founder and member of ImmunoTronics.

Rafał Derlacz, Programme Director at Polpharma Biologics, experienced project manager with a demonstrated history of working in the pharmaceuticals industry. Strong program and project management professional skilled in team building, biotechnology, market research, metabolism, and pharmaceutical industry.

D-2: Future is now – support and development of Polish biotechnology

Biotechnology is no longer the realm of science fiction — it is one of the most dynamic and transformative sectors in today's world. Gene editing, targeted therapies are becoming everyday tools in modern laboratories. This panel will focus on the future of Polish biotechnology, emphasizing the need for systemic support, effective science-industry collaboration, and favourable conditions for world-class innovation. Will Poland harness its potential and join the global biotechnology race — or be left behind as a passive observer? From our panelists, you expect insights into the current state and untapped potential of Poland's biotechnology ecosystem, how Polish innovators are contributing to global breakthroughs and open, honest dialogue with the audience.

The discussion will be moderated by **Klaudia Staśkiewicz**, who will guide the audience into the world of cutting-edge biotechnology—its promises, challenges, and the choices that will shape Poland's position on the global innovation map.

The panelists

Gajane Żurawska (Bacteromic), manager with 29 years of experience in the pharmaceutical and MedTech industries. She specializes in marketing, sales, business development, and strategic portfolio management. A pharmacy graduate, she has pursued further education in strategic management and business, both in Poland and abroad.

Maciej Gołaszewski (BioCloner Health), engineer, innovator, and R&D manager with over 10 years of experience in biomedical engineering, 3D printing, and advanced materials. He has led international projects, authored scientific publications and patents, and actively supports science-business collaboration. Currently a board member at BioCloner Health, where he oversees interdisciplinary projects aimed at improving human and animal health.

Aleksandra Izdebska earned her PhD in Analytical Chemistry at the University of Pau and Pays de l'Adour, France. Her research focuses on developing analytical methods to examine arsenic uptake and transformation in tree seedlings with phytoremediation potential. She acted as a board member of the Warsaw Society of Biothechnology "Symbioza" in 2017–2018, and remains its active member to date.

W-1: On the production line – you decide

Małgorzata Ulanowicz[1,2], *Emilia Cywińska* [1,2]

[1] Bioton

The goal of the workshop is to engage participants in realistic decision-making scenarios faced by biotech teams during the production of recombinant proteins. Participants will take on the roles of people responsible for the quality, development and safety of the product – from biosynthesis, through purification, to the to the final drug formulation. This interactive quiz stimulates strategic thinking, encourages collaboration, and demonstrates how even the smallest decision can impact patients' health and a company's success.

Available spots: 60

W-2: Design thinking in biotechnology

Wiktoria Frączek [1]

[1] Warsaw University of Life Sciences

What if innovation in biotechnology didn't begin with data or design, but with a moment of empathy?

A moment to ask not what we can build but what truly matters, and for whom. This workshop invites you to take on a real-world challenge from the field of biotechnology — something messy, complex, and unsolved, like hospital-acquired infections and antibiotic resistance. You'll step into the shoes of people affected by the problem, from healthcare workers to patients, and work in small teams to use the design thinking process to uncover human needs behind technical issues, generate bold ideas, and quickly build and share a prototype. You'll have the freedom to shape your ideas in whatever way helps them come alive and makes them visible to others. This is not about having the right answer — it's about asking better questions, thinking with your hands, and creating with purpose. You'll move fast, think hard, test ideas, and make choices, all within 90 minutes.

If you've ever felt that science could be more human, more intuitive, more connected — this space is for you.

Available spots: 16

W-3: Bioinformatics essentials: Programming the flow of genetic information

Michał Stanowski [1]

[1] University of Warsaw

The central dogma of molecular biology – despite its apparent simplicity – encompasses complex processes such as transcription, splicing, and translation. Although studied for years, these mechanisms still hold many mysteries. The same can be said for bioinformatics – a field that increasingly supports biological research but remains not fully understood by many. During this workshop, you will step by step code the processes related to the flow of genetic information using Python. You will learn the basics of programming, how to analyze protein sequences, and how to build your own phylogenetic tree. All of this will be done in a practical, accessible way – whether you already have coding experience or are just starting out.

Available spots: 16

W-4: Basics of bioprinting with BioCloner Health

Julia Talecka[1], *Jakub Knap-Wardzyński*[1]

[1]BioCloner Health

We are a company committed to improving the quality of life and health for both humans and animals by bringing innovative technologies into medical areas. During this presentation, we will showcase our software for operating the BioCloner Desktop Pro — a 3D bioprinter designed to support users throughout the entire bioprinting process. Attendees will learn how to prepare a 3D model for printing: from importing and editing the file, through setting print parameters, to selecting the appropriate bioink and printhead. We will also explain the principles of the bioprinter's operation and how it precisely deposits biological material layer by layer. Emphasis will be placed on the user-friendly interface, flexible configuration options, and the software's adaptability to various applications — from tissue models to experimental structures. This presentation is aimed at biomedical engineers, researchers, and anyone interested in cutting-edge bioprinting technologies in medicine and biology.

Available spots: 16

O-1: Bimetallic nanozymes as labels for a novel approach to signal generation in paper-based immunoassays

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In recent years, paper-based assays have become an essential part of diagnostic processes, particularly in the detection of infectious diseases, as demonstrated during the COVID-19 pandemic. However, conventional lateral flow assays (LFAs) suffer from limitations, such as low sensitivity and high detection limits. These drawbacks lead to a number of false-negative results and restrict their applicability. Nanomaterials mimicking enzymatic activity (nanozymes) offer a solution due to their superior stability and resilience (e. g. temperature and pH) compared to natural enzymes. These properties make nanozymes highly suitable for enhancing LFAs by improving detection sensitivity and lowering detection limits. While most commercially available LFAs utilize gold nanoparticles (NPs) as labels for signal generation, this approach often fails to provide sufficiently low detection limits for many disease markers. In this study, bimetallic NPs were synthesized using a chemical reduction method with polymer-based stabilizers such as poly(vinyl alcohol) (PVA). Precursor solutions of transition metal compounds, including chloroplatinic acid (H_2PtCl_6) and tetrachloroauric acid ($HAuCl_4$), were mixed in various molar ratios to form bimetallic structures. One example is gold-platinum NPs with a 1:1 molar ratio, exhibiting enzyme-like properties. The synthesized NPs were characterized using dynamic light scattering (DLS) and UV-Vis spectrophotometry to determine their physicochemical properties. Selected NPs were subsequently conjugated with mouse anti-CRP antibodies, and the prepared conjugates were incorporated into a lateral flow assay for CRP detection, utilizing nanozymes as labels. This work demonstrates that nanozymes exhibiting catalytic activity can effectively lower the limit of detection in LFAs, thereby enabling the detection of new disease markers in paper-based immunoassays. These improvements enhance the functionality and reliability of point-of-care diagnostic devices.

Keywords: nanozymes, catalytic activity, lateral flow assay, transition metals

O-2: Hybrid Magnetic-Core-Based Nanozymes: A Versatile Platform for Bioanalytical and Biomedical Applications

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The integration of diverse nanoparticles with unique intrinsic properties unlocks new opportunities for their biomedical and bioanalytical applications. Magnetic nanoparticles (MNPs) serve as an excellent foundation for constructing multifunctional nanostructures. Although not inherently magnetic, they can be efficiently manipulated under external magnetic fields, facilitating targeted delivery and effective separation.

In this study, MNPs were decorated with catalytic nanoislands of high catalytic activity and further functionalized with polyphenols via their polymerization. The resulting polypolyphenol layer acted as stabilizing agent while enhancing biocompatibility, optical properties, and surface reactivity. The use of naturally derived polyphenols improved the nanostructures adaptability for biomedical applications. Two types of MNPs were used: Fe_3O_4 synthesized via controlled oxidation and Fe-Co alloyed nanoparticles obtained via hydrothermal method. Noble metal nanoislands were introduced to anchor and catalyze polyphenol polymerization under varying reaction conditions (pH, oxidizing agents).

Characterization of the resulting nanostructures revealed enhanced optical properties, including increased absorbance in the visible and near-infrared (NIR) range. These improvements position them as promising candidates for optical biosensing. Their heightened NIR absorbance also suggests potential for photothermal anticancer therapy, where efficient light-to-heat conversion is critical. Additionally, the polypolyphenol coating offers abundant functional groups, facilitating biomolecule attachment for selective and sensitive biosensor development. This adaptable platform allows for precise customization, supporting advancements in diagnostics, targeted therapy, and environmental monitoring. The multifunctionality of these hybrid nanostructures highlights their broad potential for both research and clinical applications.

IDUB POSTDOC VI (CPR-IDUB/310/Z01/Z10/2024)

Keywords: magnetic nanoparticles, nanozymes, polyphenols, photothermal therapy

O-3: Theranostic properties of oxygen nanobubbles

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Hypoxia is a typical characteristic of the tumor microenvironment in cancer. Low oxygen levels can reduce the effectiveness of conventional cancer treatments. Oxygen nanobubbles are emerging as a promising therapeutic option, as they release oxygen locally when exposed to ultrasound pulses through cavitation. Both microbubbles and nanobubbles are already used as efficient contrast agents in ultrasound imaging. To investigate their potential in theranostic applications, experiments were conducted using phantom models and a 4T1 breast cancer model implanted in the mammary fat pad of Balb/c female mice. The goal of this study was to assess the contrast properties of oxygen nanobubbles, evaluate their response to ultrasound, and determine if they could effectively increase oxygen partial pressure in animal tissues. The initial phase of the study focused on determining the appropriate ratio of oxygen and perfluorocarbon content in the gas core of the bubbles and evaluating their stability using ultrasound imaging for that purpose. Dynamic light scattering (DLS) measurements, which were performed to determine the size of ONBs, showed that the average vesicle size remained under 400 nm for up to 60 minutes post-activation, suggesting their suitability for in vivo use. Additional analysis with a nanoparticle tracking analyzer (NTA) revealed a decrease in nanobubble size and concentration after ultrasound exposure. Further tests confirmed that the nanobubbles enhanced contrast during ultrasound imaging, leading to a significant increase in the recorded signal. Finally, *in vivo* EPR-based oxymetric measurements showed a substantial improvement in tissue oxygen levels in the animals.

The work was financed by the OPUS Project, 2022/45/B/NZ4/01215, National Science Center.

Keywords: Oxygen, Nanobubbles, Ultrasounds, Ultrasonography, EPR

O-4: Sponge-like titania nanotubes with M13 bacteriophages for *Escherichia coli* detection

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M13 bacteriophages (in short, phages) have an intrinsic ability to infect *Escherichia coli* (*E. coli*) bacteria. They can also be engineered to exhibit specific peptides on their surface enabling phages to selectively bind to diverse targets (Janczuk-Richter et al. 2019). Therefore, both modified and wild-type phages can be used as a sensing element in a biosensor. Consequently, our aim is to assess the effectiveness of sponge-like, laterally spaced, hydrogenated titania nanotubes (S-TiO_2-NTs) as an immobilisation platform for M13 phages in electrochemical systems. The electrodes used in this study were fabricated via anodization and calcination in a hydrogen atmosphere (Lipińska et al. 2024). Electrochemical methods were used to study the capacitive and Faradaic currents of the S-TiO_2-NTs electrodes in varying concentrations of M13 phage lysate immobilised through physisorption. Furthermore, measurements at 37°C and in human serum were conducted to evaluate the stability of the studied electrodes. The obtained results suggested that M13 phages were successfully adsorbed on the surface of the electrodes. These findings were confirmed by scanning electron microscopy analysis. Finally, the S-TiO_2-NTs electrodes modified with wild-type phages were utilized for *E. coli* bacteria detection. The limit of detection for the electrodes equals 3 cells/ml, and the linear range was 10-10^4 cells/ml. Additionally, measurements with bovine serum albumin as a blocking agent were performed. The results indicate that the M13 phages can be successfully immobilised by physisorption on the S-TiO_2-NTs electrodes making them potentially valuable in biosensing applications.

The financial support was given from National Science Centre via project OPUS LAP 2020/39I/ST5/01781 for Katarzyna Siuzdak and project SONATA 2017/26/D/ST5/00980 for Katarzyna Szot-Karpińska.

[1] Janczuk-Richter M. et al. (2019) Recent applications of bacteriophage-based electrodes: A mini-review. Electrochemistry Communications 99: 11-15.
[2] Lipińska W. et al. (2024) Coupling between the photoactivity and CO2 adsorption on rapidly thermal hydrogenated vs. conventionally annealed copper oxides deposited on TiO2 nanotubes. Journal of Materials Science 59: 16947-16962.

Keywords: bacteriophage, titania nanotubes, surface modification, bacteria detection, electrochemistry

O-5: Illuminating the Unseen: Microbial Rhodopsins in Freshwater Lakes

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Microbial rhodopsins are light-sensitive proteins that perform various functions, including ion pumping and phototaxis sensing. Their early evolutionary emergence has resulted in a remarkable diversity. By converting light energy into chemical energy, rhodopsins play a critical role in energy processes and hold significant potential for biotechnology and medicine. They are particularly promising for applications in bioenergy production, optogenetics, and the study of neurodegenerative diseases, offering new ways to interact with living cells using light. To fully exploit these possibilities, global studies on rhodopsin diversity are essential. Metatranscriptomics has become a crucial tool for discovering new rhodopsin types, enabling the analysis of gene expression across entire microbial communities, including in less-studied freshwater environments. Despite extensive research on marine rhodopsins, freshwater ecosystems, particularly in relation to eukaryotic microorganisms, remain underexplored. This study aimed to conduct a metatranscriptomic analysis on samples from Masurian lakes to identify rhodopsins in eukaryotic microorganisms. A total of 1,153 rhodopsin sequences were detected across all samples. Additionally, the identified rhodopsins were taxonomically annotated, and the presence of additional functional domains was also determined. An optimized bioinformatics pipeline was also developed, using Trinity, MegaHIT, and rnaSPAdes for metatranscriptome assembly and data clustering. The effectiveness of these programs was evaluated based on sequence quality, computational efficiency, and analysis time. The results revealed a significant number of unique rhodopsin sequences in dystrophic lakes, suggesting the specific environmental characteristics of these ecosystems. These findings underscore the need for further research to better understand the role of rhodopsins in freshwater ecosystems and their potential for biotechnological applications.

Keywords: rhodopsins, metatranscriptomics, bioinformatics, retinal, eukaryotic microorganisms

O-6: A bone to pick with ancient DNA! Evaluating auditory ossicles as a new resource for genetic studies.

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A key challenge in ancient DNA research is the limited amount of endogenous genetic material recovered from skeletal remains. In archaeogenetic analyses, less than 10% of DNA sequences retrieved are typically from endogenous sources. The preservation of human DNA varies depending on the bone tissue selected, and identifying optimal sources can improve the proportion of endogenous DNA and the quality of the extracted material. In this study, we examined not only the commonly used teeth and inner ear fragments from the petrous temporal bone but also auditory ossicles, which have recently been proposed as a potential source of ancient DNA. Unlike existing methods, we standardized the extraction procedure across all tissue types by using dissolution instead of mechanical homogenization. Additionally, we assessed the impact of excluding heat and friction, which are usually caused by mechanical homogenization, on the quantity of endogenous DNA recovered. We will continue to investigate metagenomic profiling of these methods, samples, and their effect on aDNA ratio and complexity. Our results show variability in the amount of endogenous DNA recovered, influenced by tissue type and the inclusion of mechanical homogenization. We argue that these factors and varying degrees of skeletal damage caused by different methods should be carefully considered when designing ancient DNA studies involving precious human bone samples.

Polish National Science Center, Grant/Award numbers: UMO-2020/39/B/HS3/00159 and UMO-2022/47/I/HS3/02274

[1] Sirak K. et al. (2020) Human auditory ossicles as an alternative optimal source of ancient DNA. Genome Research 30: 427-436.

Keywords: Ancient DNA, Auditory ossicles, material comparison, Archaeogenetic analyses, dissolution

O-7: High-Throughput Analysis of Chloroplast tRNA Modifications and Their Changes Under High Light Stress

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Chloroplasts are semi-autonomous organelles responsible for photosynthesis and cellular metabolism in plants, algae, and some protists, making them the primary source of chemical energy on Earth. They likely originated from an ancient photosynthetic bacteria through endosymbiosis, retaining their own genome and translational machinery. Transfer RNAs (tRNAs) are critical for chloroplast translation, with chemical modifications influencing stability, structure, and translational efficiency. These modifications may influence translation, particularly in response to stress conditions. Our research focuses on characterizing the chloroplast tRNA expression and modification landscape, and its alterations under high light (HL) stress. Given that oxidative stress in bacteria influences tRNA modifications for cellular adaptation, we hypothesize that a similar mechanism occurs in chloroplasts in various stresses that lead to ROS (reactive oxygen species) production, such as HL stress. To investigate this, we optimized a high-throughput sequencing-based approach to establish a reference profile of chloroplast tRNA modifications under control conditions. We observed similarities to bacterial tRNAs, particularly in anticodon and variable loops, along with structural features resembling those in eukaryotic systems. Additionally, we identified unique modifications likely contributing to chloroplast-specific translation regulation. Currently, we are analyzing how HL affects the chloroplast tRNA expression and modification landscape. Our findings reveal shifts in chloroplast codon preferences, where codons preferred under control conditions become even more favored under stress. This supports the hypothesis that tRNA modifications fine-tune gene expression by modulating translation efficiency in response to environmental conditions. Our study provides new insights into the adaptive role of tRNA modifications in chloroplast function and plant stress tolerance.

This work is part of the Sonata Bis 11 project titled T̈he role of tRNA expression and modification in chloroplast translation during stress (UMO-2021/42/E/NZ3/00274);̇ funded by the National Science Centre (NCN).

Keywords: tRNA, modifications, chloroplast, high light

O-8: Unraveling the role of eriophyoid mites in the spread of raspberry leaf blotch emaravirus (RLBV). Implications for virus transmission and control

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Raspberry leaf blotch emaravirus (RLBV, *Emaravirus idaeobati*, family *Fimoviridae*) is among the most common *Rubus* viruses affecting raspberry plantations across Europe. It negatively affects plant growth and fruit quality, reducing raspberry yields [1]. Poland is one of the largest berry producers in the world; therefore, a better understanding of RLBV and improved virus eradication efforts are crucial for domestic berry growers. It has been long suspected that the raspberry leaf and bud mite (*Phyllocoptes gracilis*) is involved in the natural spread of RLBV; however, transmission tests conducted for this species were inconclusive [2]. This study aimed to (A) identify the fauna of eriophyoid mites present in raspberries and blackberries in Poland, (B) conduct RLBV transmission tests with mite species or genotypes predominant on infected plants, (C) examine the possibility of RLBV replication within the body of a vectoring mite. For this purpose, we have collected 200 samples in Poland, both from cultivated and naturally growing raspberry and blackberry plants. Samples were tested for RLBV using RT-PCR, with 26 raspberries and one blackberry being infected. Barcoding of mites and phylogenetic analysis revealed two species of eriophyoids being associated with raspberry, whereas only one species was present on blackberry plants. RLBV transmission tests unequivocally confirmed vector-competence of *P. gracilis*, and virus quantification assay within individual mites provided a new perspective on RLBV accumulation and retention. Identification of RLBV vector and improved understanding of transmission mechanisms are crucial for developing effective control strategies that could be used in the field.

Understanding the molecular and ecological interactions between viruses and eriophyoid mites 2021/43/P/NZ9/03267

[1] Gritsenko D. et al. (2022) Development of primer sets for detection of Raspberry leaf blotch virus and Raspberry leaf mottle virus by multiplex RT-PCR. Eurasian Journal of Applied Biotechnology 1: 33–39.
[2] McGavin W. et al. (2012) Raspberry leaf blotch virus, a putative new member of the genus Emaravirus, encodes a novel genomic RNA. Journal of General Virology 93: 430-437.

Keywords: Mites, RLBV, Rubus, Phylogenetics, DNA barcoding,

O-9: UPCYCLING SPENT COFFEE GROUNDS: A NATURAL SOURCE OF ANTIOXIDANTS AND OILS FOR COSMETICS

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Coffee is one of the most widely consumed beverages in the world, with coffee bean consumption generating millions of tons of spent coffee grounds (SCG) annually. A significant share of this waste ends up in landfills, posing environmental concerns. However, despite the brewing process, SCG remains a rich source of bioactive compounds, including lipids and antioxidants, which can be extracted as coffee oil and antioxidant extracts. These compounds exhibit properties that may benefit skin health, making them promising candidates for cosmetic applications. This study explores the potential use of SCG-derived ingredients in cosmetic emulsions.

O/W emulsions with various concentrations of SCG-derived ingredients were prepared by hot emulsification processing. The physical stability of the creams was studied in the accelerated aging test. The influence of temperature on the antioxidant activity of coffee oil and antioxidant extracts was assessed by the CUPRAC assay. The cytotoxicity of individual SCG-derived ingredients as well as the emulsions containing them was tested via FDA/PI and MTT assays on human keratinocytes from the HaCaT cell line in a 2D and 3D culture.

The addition of coffee oil and antioxidant extracts to the emulsions did not cause mixing difficulties, changes in consistency or pH. After the accelerated aging test there were no signs of emulsion delamination. The antioxidant activity of SCG-derived ingredients was not affected by the temperature. Coffee oil and antioxidant extracts showed no cytotoxicity towards human keratinocytes in a monolayer and spheres. Similar results were obtained for emulsions containing SCG-derived ingredients.

Spent coffee grounds are a rich source of antioxidants and coffee oil, with potential applications in the cosmetic industry. The SCG-derived products analyzed in this study were proved to be suitable as active and base ingredients for cosmetic emulsions.

Keywords: spent coffee grounds, antioxidants, coffee oil, cosmetic emulsions

O-10: Semi-synthetic ceramides influence on skin cell viability and cellular behavior in 2-dimensional (2D) and 3-dimensional (3D) models

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Ceramides are lipids built of sphingosine and fatty acids. Situated in the *stratum corneum*, they are arranged into multilamellar membranes and thus prevent water loss and/or penetration of alien substances. Moreover, ceramides influence various processes such as apoptosis, proliferation, differentiation, or inflammation [1-2]. Most studies on synthetic ceramide properties are conducted on 2D models, which do not reflect interactions occurring in the human body. In this study, we aim to compare the effects of selected ceramides on the viability and behavior of skin cells in 2D and 3D models. The study was conducted using immortalized human keratinocytes (HaCaT). Initially, the influence of semi-synthetic ceramides III, IIIB, sphingoceryl, and phytosphingosine was assessed using tetrazolium salt (MTT), fluorescein diacetate/propidium iodide (FDA/PI), and resazurin assays. The ceramide influence was compared in the 2D and 3D (spheres) models. The impact of ceramides on cell adhesion to extracellular matrix (ECM) proteins and the ability to aggregate were investigated by cell-substrate adhesion assay as well as sphere formation assay, respectively. Finally, the influence of treatment on cell migration was estimated using scratch assay. The obtained results indicate that the selected ceramides decrease keratinocyte viability in both 2D and 3D models. However, in the case of the 3D model, the effect was weaker than in the 2D models. The influence of ceramide III and IIIB on the skin cells was similar, while phytosphingosine showed a stronger cytotoxic effect. The influence of sphingoceryl was comparable to phytosphingosine. The cell-substrate adhesion to ECM proteins and migration of cells treated with all tested ceramides were inhibited, but the treatment did not affect cell aggregation. In conclusion, tested semi-synthetic ceramides decreased the viability of keratinocytes in 2D models more noticeably than in 3D models, as well as significantly changed cellular behavior.

This research was funded by the Warsaw University of Technology within the Excellence Initiative: Research University (IDUB) programme in the form of YOUNG PW II project.

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[2] Li Q. et al. (2020) The role of ceramides in skin homeostasis and inflammatory skin diseases. Journal of Dermatological Science 97: 2-8.

Keywords: epidermis, keratinocytes, ceramides, 3D models, spheres

O-11: Spent coffee grounds as a source of antioxidant extract with caffeine and phenolic acids with potential effects on skin cells

Adrianna Piasek[*,1], *Zoja Trojan*[1], *Małgorzata Zduńczyk*[2], *Karolina Jelonek*[3], *Paula Bardadyn*[3], *Tomasz Kobiela*[2], *Anna Sobiepanek*[2]

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Coffee waste serves as a rich and sustainable source of bioactive compounds, including cellulose, proteins, and oils, with polyphenols standing out for their antioxidant properties. This research focuses on extracting an antioxidant fraction from spent coffee grounds, containing caffeine and phenolic acids such as chlorogenic acid and caffeic acid. The extract was analyzed for its composition and evaluated for its biological effects on both normal and cancerous skin cells. To optimize bioactive compound yield while minimizing degradation, the extraction process was carefully refined. High-performance liquid chromatography was employed to identify key polyphenols. The antioxidant capacity of the extract was assessed using DPPH, FRAP, CUPRAC, ABTS assays, and also by the Total Polyphenol Content. *In vitro* studies on human skin cells, both healthy and cancerous, were conducted to assess cytotoxicity (MTT, FDA, PI), collagen production, reactive oxygen species (ROS) generation of the extract and its components. The results demonstrated that the antioxidant extract provided protective effects against oxidative stress in normal skin cells, while caffeine and chlorogenic acid induced apoptosis in skin cancer cells. These findings highlight the dual role of SCG polyphenols in promoting skin health emphasizing the potential of upcycling spent coffee grounds into valuable bioactive extracts.

This work was supported by the National Science Centre (Poland) Grant no. 2023/49/N/NZ5/03578 and Implementation PhD edition V financed by Ministry of Science and Higher Education.

Keywords: antioxidants, spent coffee grounds, skin cells, skin cancer

O-12: Unveiling the Secrets of Melanogenesis: Depigmenting Agents in Melanoma

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Melanogenesis is a complex process of melanin production, the pigments responsible for the coloration of the skin, hair, and eyes, involving several types of skin cells, with melanocytes playing the most crucial role. Disruptions in the melanogenesis process can lead to various pigmentation disorders. Currently, different depigmenting agents are used, such as hydroquinone, retinoids (vitamin A or tretinoin), and azelaic acid. However, their use is associated with side effects, including skin irritation, allergic reactions, and, in the case of hydroquinone, potential toxicity risks. The aim of the conducted research was to explore the impact of several depigmenting agents (vitamin C, kojic acid, phenylthiocarbamide) on melanoma cell lines and the process of melanogenesis. The studies were conducted on human (MeWo, WM266-4, G-361) and murine (B16-F10) melanoma cell lines. Initially, the cytotoxic effects of selected compounds on the cells were examined. Additionally, the expression of genes associated with melanogenesis in the human cell lines used in the research was analyzed. Subsequently, the impact of these compounds on melanogenesis inhibition in the mentioned cell lines was evaluated. Furthermore, their antioxidant properties and potential to reduce reactive oxygen species (ROS) production by cells upon exposure to these compounds were assessed. A protocol for melanogenesis stimulation was also developed for the MeWo cell line, which is incapable of spontaneous pigmentation under *in vitro* culture conditions. The protocol for the assay directly measuring melanin content in pigment-producing cells was optimized. The tested compounds did not exhibit any negative effects on the viability of human and murine melanoma cell lines. These compounds demonstrated antioxidant properties and inhibited ROS production by cells. Among the analyzed human cell lines, the highest expression levels of melanogenesis-related genes were observed in MeWo cells.

Keywords: melanogenesis, vitamin C, kojic acid, phenylthiocarbamide

O-13: BrewCure — From Waste to Wellness: Unveiling the Biological Potential of Arabica and Robusta Coffee Waste Extracts

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With the global surge in coffee consumption, spent coffee grounds (SCG) have become an abundant yet underutilized waste product. Studies indicate that SCG extracts exhibit significant antimicrobial and antioxidant activities, making them a valuable resource for biomedical applications. This study investigates the antimicrobial, antifungal, and antioxidant properties of ethanolic extracts derived from Arabica and Robusta coffee waste. We systematically evaluated their efficacy against pathogenic bacteria (*Staphylococcus aureus, Listeria monocytogenes,* and *Escherichia coli*) and fungi (*Candida albicans*), demonstrating significant inhibitory effects. Additionally, antioxidant potential of the extracts was assessed using DPPH, FRAP, and CUPRAC assays; while cellular studies on human keratinocytes provided insights into their protective role against oxidative stress. These findings highlight distinct bioactive profiles between Arabica and Robusta SCG extracts, positioning them as valuable candidates for sustainable antimicrobial and dermatological applications. By transforming coffee waste into a functional resource, this research aligns with circular economy principles and advances the development of eco-friendly biotechnological solutions. Furthermore, these insights contribute to the growing field of natural compound-based therapeutics, opening new pathways for innovative applications in medicine and industry.

This research was funded by the Rector's Grant of the Warsaw University of Technology under the Excellence Initiative - Research University program.

Keywords: Spent coffee grounds, antioxidant activity, antimicrobial properties, waste valorization, Arabica, Robusta, circular economy, biomedicine

O-14: The impact of pollution on the electrophysiology of epithelium - observations from Caco-2 cell model

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Nowadays, the problem of plastic pollution and its impact on living organisms has become a critical environmental concern. The breakdown of plastic debris produces micro- and nanoparticles which are ingested by living organisms and interact with the intestinal barrier. However, our knowledge of their effects on human epithelial tissues as well as on transepithelial water and ion transport remains inadequate. The aim of this study was to examine the influence of polystyrene nanoplastics: PS-NPs (100 nm diameter) on the human intestinal epithelial cell line Caco-2. This research focused on the observed increased mucus secretion displayed by Caco-2 cells in response to PS-NPs treatment. Utilizing Ussing chamber studies, we deduced that PS-NPs alter ion transport across epithelial cell monolayers. The presence of nanoplastics decreased CFTR channel activity, however, increased the activity of CaCC channels, e. g. TMEM16a, which is responsible for healthy mucus production. Its role in observed mechanism was verified by ion transporting proteins modulators and calcium release indicator. The cytotoxic potential of PS-NPs and its influence on Transepithelial Electrical Resistance (TEER) was also shown. This research validates that the elevated TMEM16a activity was responsible for the observed increased mucus secretion, acting as a recently discovered defence mechanism of Caco-2 cells against PS-NPs.

This work was supported by the System Wsparcia Finansowego SGGW -SWF/2/2024 (MZ) and by the National Science Center (NCN) Poland no. 2024/53/B/NZ3/01635 (PB).

Keywords: Polystyrene, Nanoplastic, Electrophysiology, Ion transport, Intestinal Epithelium

O-15: Heme oxygenase-1 affects nuclear envelope structure

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Heme oxygenase-1 (HO-1, *Hmox1*) degrades excess heme, protecting cells from oxidative and replication stress. Recently, we observed a decreased expression of *Lmna* (lamin-A/C) in Hmox1-deficient cells. Here, we aimed to check whether such a decrease is associated with any functional effects and to identify possible underlying mechanisms.

We used murine fibroblasts (*Hmox1*$^{+/+}$ and *Hmox1*$^{-/-}$) and iPS cells lacking both HO-1 and HO-2 or possessing only HO-1 localized either in the cytoplasm or the nucleus. Using atomic forces microscopy, we observed increased nuclear elasticity in the absence of *Hmox1*, along with a reduced nuclear area and height. Scanning electron microscopy further demonstrated that *Hmox1*-deficient cells exhibit a flattened shape, particularly in the nuclear region. We also detected the exonuclease TREX1 within the nuclei, and elevated DNA levels in the cytoplasm of such cells. Both observations suggest an impairment in nuclear envelope integrity in the absence of HO-1. Additionally, in *Hmox1*$^{-/-}$ fibroblasts, we observed a decreased nuclear localization of p53, while proximity ligation assays (PLA) revealed reduced colocalization between lamin-A and PARP1. It seems important, as both PARP1 and p53 regulate *Lmna* expression and lamin A stability. Indeed, our preliminary data show a decrease in lamin A level in p53-deficient fibroblasts or in cells with inhibited PARP1 activity. Moreover, the lack of Hmox1 is accompanied by changes in the PARP1 interactome, including increased colocalization with p53. Previously, we revealed that HO-1 supports p53 activity via the regulation of heme, the trigger of p53 degradation. Our preliminary results suggest, however, that the heme-p53 interaction is not a mediator of the Hmox1-dependent effect on lamin A.

To sum up, we demonstrated that HO-1 is needed for nuclear envelope integrity. The proper activities of p53 and PARP1 are upstream regulators of lamin A. We are currently working to clarify the underlying mechanism(s).

The study was partly financed by the ID.UJ grant: Student's research projects 19000082_N_25_40.

Keywords: heme oxygenase-1, nuclear envelope, p53, PARP1

O-16: GYY4137, a slow-releasing hydrogen sulfide donor attenuates fibrosis and inflammation in the diaphragm of dystrophic D2.*mdx* mice

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Duchenne Muscular Dystrophy (DMD) is a genetic disorder associated with the X chromosome, affecting approximately 1 in 5,000 male births. It is caused by a mutation in the dystrophin gene, located on the short arm of the X chromosome, which disrupts the reading frame and results in the production of a non-functional protein that undergoes degradation. Its absence triggers an immune response, leading to immune cell infiltration, muscle tissue damage and progressive muscle degeneration. Ultimately, the loss of diaphragm function, the primary muscle responsible for breathing, results in respiratory failure and, eventually, premature death. Our recent studies demonstrated that sodium hydrosulfide (NaHS), a fast-releaser of hydrogen sulfide (H_2S), can mitigate disease symptoms in the *mdx* mice, a commonly used DMD model [1]. In the present work, we investigated the effects of GYY4137, a slow-releasing H_2S donor, in D2.*mdx* mice, a more severe DMD model. Histological analysis using hematoxylin and eosin (H&E) staining revealed that H_2S donor administration reduced immune cell infiltration, myofiber swelling, and rhabdomyolysis in the diaphragm muscle. Additionally, gene expression analysis of profibrotic markers (*Col1a1* and *Col3a1*), along with Masson's trichrome staining, demonstrated the anti-fibrotic properties of GYY4137 treatment. Furthermore, imaging of Sirius red staining under polarized light showed a reduced area occupied by collagen deposits in the diaphragm tissue. These findings suggest that hydrogen sulfide alleviates inflammation and fibrosis in D2.*mdx* mice, highlighting its potential as a therapeutic strategy for DMD.

This work was supported by grant #2019/35/B/NZ3/02817 (to AŁ) from the National Science Centre.

[1] Myszka M. et al. (2023) Sodium hydrosulfide moderately alleviates the hallmark symptoms of Duchenne muscular dystrophy in *mdx* mice. European Journal of Pharmacology 955: 175928.

Keywords: Duchenne muscular dystrophy, hydrogen sulfide donor, GYY4137, muscle fibrosis and inflammation

O-17: SUB-MIC ANTIBIOTIC EXPOSURE PROMOTES HETERO-RESISTANCE IN BACTERIA

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Background: Bacterial cells present different phenotypes even within monoclonal populations, conferring them selective advantages during stress. On antibiotic exposure, if the individual cells show differential minimum inhibitory concentrations (MICs), they are called hetero-resistant which plays a major role in selection, evolution and resistance in bacteria. Action of an antibiotic is accompanied by a variety of responses in bacteria, which cumulatively inhibit their growth. However, inefficient exposure might lead to change in the cells. Such changes may alter the response on subsequent exposure against the same or a different antibiotic, including hetero-resistance pattern. During antibiotic susceptibility testing (AST), a term single cell MIC (scMIC) is introduced which is the population average level of concentration inhibiting the proliferation of individual cells. The probability distribution of scMIC gives an insight into the hetero-resistance of the population. Methods: Droplet microfluidics is widely used in AST to separate individual cells via stochastic confinement. Overnight cultures of bacteria were refreshed till OD 0.1-0.2, exposed at sub-MIC till OD 0.1-0.2, encapsulated 1 cell per 1nL droplet, incubated for 15 hours, images acquired, analyzed, scMIC determined, resistance profile and probability distribution plotted. Results: Data analysis revealed differences among droplet intensities even within one concentration of antibiotic which directly correspond to how individual cells grew inside one droplet. The scMIC distribution also changed for the cells exposed with antibiotic and the pattern depended on the antibiotic used for exposure as well as re-exposure. Conclusions: What doesn't kill you makes you stronger- this line perfectly fits in our study where the bacteria stimulated with low antibiotic concentration showed increased survival. Antibiotics targeting the nucleotide and protein machinery were most potent to alter the hetero-resistance. Further studies will establish pattern, based on antibiotic class and mechanism of action. The data from the study can be very crucial to better the resistance mechanism, thereby helping in its efficient management and antibiotic development.

We acknowledge National Science Center, Poland and Foundation for Polish Sciences for the necessary finances. We also acknowledge Institute of Physical Chemistry of PAS for necessary support.

Keywords: Microfluidics, Antibiotic resistance, single cell, heteroresistance

O-18: Relation between body mass and vaginal microbiome of patients with polycystic ovarian syndrome diagnosis

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Polycystic ovarian syndrome (PCOS) is an endocrine disorder often linked to metabolic disturbances. Hormonal imbalances disrupt the menstrual cycle, which eventually results in infertility [1]. Emerging evidence suggests that the vaginal microbiome plays a crucial role in reproductive and metabolic health [2]. However, little is known about how BMI influences the vaginal microbiome in PCOS patients. This study aimed to examine the vaginal microbiome of patients with PCOS. We analyzed the microbiome profiles of 55 individuals based on complete 16S rRNA gene sequences obtained by Nanopore sequencing technology. For simplicity, patients were categorized by body mass index (BMI) as two groups: normal (BMI <25; n=25) and overweight (BMI 25<; n=30). As a result, alpha diversity comparison between diagnoses confirmed similarity in vaginal microbiomes. However, grouping patients by BMI demonstrated higher vaginal microbiome diversity in overweight women than those with normal BMI. We performed an analysis of differential abundance. The discovered bacteria specific for the vaginas of PCOS overweight women were taxa of increased abundance: *Anaerococcus prevotii, Anaerococcus mediterraneensis, Dialister* sp. , *Streptococcus thermophilus, Clostridiales difficile, Campylobacter hominis, Fusobacterium nucleatum, Streptococcus suis, Campylobacter ureolyticus,* and *Streptococcus pyogenes*. In contrast, the only identified taxon of decreased abundance was *Roseburia hominis*. The list of species was recognized as of high statistical significance (p-values < 0.0001). In conclusion, our findings recognized that overweight PCOS patients exhibit higher microbial diversity in the vagina. The predominance of taxa gains in higher BMI individuals suggests a potential link between metabolic status and vaginal microbiota alterations. These insights may contribute to the understanding of microbial influence on PCOS and metabolism, warranting further investigation into their clinical implications.

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[2] Pereira M., Jones S., Costin J. (2024) Association of Polycystic Ovarian Syndrome (PCOS) With Vaginal Microbiome Dysbiosis: A Scoping Review. Cureus 16: e62611.

Keywords: body mass, gut microbiome, polycystic ovarian syndrome, vaginal microbiome

O-19: 4 types of healthy uterus microbiome

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Microorganisms habituate the female genital tract and consist of up to 9% of the human total microbiome. Uterine microbiota is 100 to 10,000 times lower in biomass than its vaginal counterpart. Different studies report *Lactobacillus* in a healthy uterus, but relative abundances vary - from over 90%, 71% to 30.6% [1]. Verstraelen et al. identified *Firmicutes* phylum as dominant in only 4 out of 19 samples and alternatively proposed *Proteobacteria*, and *Bacterioidetes* as dominant taxa [2]. Sola-Levya et al. identified *Klebsiella pneumoniae* and *Clostridium botulinum* as the most frequently recovered bacteria in the endometrium, which pathogenicity is bothering. The lack of consistency regarding predominant bacteria and their impact on the healthy uterus demonstrates the necessity of expanding the research.

This study aimed to examine the composition of bacteria in a healthy uterus. We analyzed the microbiome profiles of 22 individuals based on complete 16S rRNA gene sequences obtained by Nanopore sequencing technology. As a result, we concluded that there are four microbiome composition types in healthy uteruses. The 1st (*Lactobacillus*-dominant), the 2nd (*Faecalibacterium*-dominant), the 3rd (*Streptococcus*-related), and the 4th (various taxa-dominant) with *Megasphaera* and *Dialister* coexistence, or *Escherichia coli* domination.

Our study revealed the importance of broader perspectives and precision at the individual level in analysis to detect any microbiome coexisting pattern. We proved that even healthy groups born in one region differ in the case of microbiome composition in the endometrium. Current results expose the diversity of bacteria taxa in the uterus, whose activity is worth further investigation. Achieved conclusions encourage the idea of dynamic relations between species in the niche and within the microbiome community and endometrial cells.

[1] Chen C. et al. (2017) The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. Nature Communications 8: 875.
[2] Verstraelen H. et al. (2016) Characterisation of the human uterine microbiome in non-pregnant women through deep sequencing of the V1-2 region of the 16S rRNA gene. PeerJ 4: e1602.

Keywords: uterus, microbiome, nanopore, bacteria

O-20: Substrate geometry affects population dynamics in a bacterial biofilm

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Biofilms inhabit a range of environments, such as dental plaques or soil micropores, often characterized by non-even surfaces. However, the impact of surface irregularities on the population dynamics of biofilms remains elusive, as most experiments are conducted on flat surfaces. Here, we show that the shape of the surface on which a biofilm grows influences genetic drift and selection within the biofilm. We culture *Escherichia coli* biofilms inside microfluidic devices in microwells with a corrugated bottom surface and observe the emergence of clonal sectors whose size corresponds to that of the corrugations, despite no physical barrier separating different areas of the biofilm. The sectors are remarkably stable and do not invade each other; we attribute this stability to the characteristics of the velocity field within the biofilm, which hinders mixing and clonal expansion. Using a mixture of antibiotic-sensitive and antibiotic-resistant mutants in the presence of sublethal concentrations of the antibiotic rifampicin, we also show that clonal expansion is limited even for clones with a significant growth advantage. Surface corrugations act thus as a suppressor of selection in the biofilm. Our results show that biofilm population dynamics can be affected by patterning the surface and demonstrate how a better understanding of the physics of bacterial growth can be used to control microbial evolution.

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[1] Postek W. et al. (2024) Substrate geometry affects population dynamics in a bacterial biofilm. Proceedings of the National Academy of Sciences 121: e2315361121.

Keywords: biofilm, bacteria, microfluidics, E.coli, population dynamics, antibiotic resistance, physics

O-21: Regulation of Hematopoietic Stem Cell Differentiation by the Neogenin-1/Netrin-1 Axis

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Hematopoietic stem cells (HSCs) can form all types of blood cells. They residue mostly in bone marrow of long bones but can also be found in peripheral and cord blood. Thanks to their ability to self-renew, the pool of HSCs is sustained during aging. HSCs can differentiate into 2 separate lineages: myeloid and lymphoid. However, with age, both the differentiation and the self-renewal of HSCs is altered. Aged HSCs have a higher potential to differentiate into myeloid cells than lymphoid cells. This process is called myeloid bias. The mechanism of these changes is not fully understood, but it is suggested that both intrinsic and extrinsic factors play a role. The aim of this study was to investigate the role neogenin-1 (Neo-1)/netrin-1 (Ntn-1) axis in the regulation of differentiation of HSCs during aging. Single Neo-1^{+} and Neo-1^{-} HSCs from young and old mice were sorted per well, where half of them were stimulated with Ntn-1 and undergo in vitro differentiation for 3 weeks. Each week colonies were examined by taking images to see possible changes during cell culture. After 3 weeks, the cells were analyzed using flow cytometry, which showed a high clonal variability within immature cells (c-kit^{+}) across all groups, as well as a higher occurrence of progenitor cells in mice expressing Neo-1. Additionally, it was observed that colonies from old mice are smaller, but the group of old mice with Neo-1 expression has the highest number of HSC colonies.

Keywords: Hematopoietic stem cells, neogenin-1, netrin-1, myeloid bias

O-22: Stroma-driven horizontal transfer of TCA proteins enhances metabolic plasticity and promotes imatinib resistance in chronic myeloid leukemia

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Bone marrow microenvironment (BMM) is shown to increase resistance of leukemic cells to tyrosine kinase inhibitors (TKIs). Emerging evidence highlights the crucial role of metabolic remodeling in this process. Here, we investigated the role of BM-stromal cells in metabolic rewiring in chronic myeloid leukemia (CML), and its role in TKI resistance.

In our CML-BMM model (K562-HS5 coculture), we analyzed oxidative metabolism changes via high-resolution respirometry and Seahorse Technology. Using a trans-well system (TW) and conditioned medium (CM), we assessed the need for direct cell-to-cell contact. Stromal mitochondria's role was tested by mtDNA depletion in HS5. Combining proteome trans-SILAC and metabolomics, we examined TNT-carried membrane vesicles' cargo from stromal donor to leukemic acceptor cells. Also, primary material was analyzed with the novel CENCAT method.

We showed that in coculture, stromal cells transfer membrane vesicles containing TCA cycle proteins and metabolites to CML cells, enhancing their metabolic plasticity. TW and CM setups confirmed the importance of TNT-mediated transfer. The condition of stromal mitochondria was irrelevant to induce stroma-driven effects. Stromal component increased TCA cycle and oxidative phosphorylation activity in CML cells, protecting them from metabolic homeostasis disturbance under imatinib (first-line TKI) treatment. CENCAT showed a similar trend in primary material. Trans-SILAC and metabolomics concurred elevated TCA cycle-related metabolites and proteins. We additionally provided dual evidence for the transfer of a potentially crucial TCA cycle protein from stromal to CML cells.

Previously, leukemic stem cells were described to undergo metabolic remodeling. Here, we show such changes as BM-driven, TNT-mediated increase in metabolic plasticity in CML cells. We suggest that TCA cycle-related metabolic plasticity in CML can be used as a target for overcoming the resistance caused by the BMM.

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Keywords: CML, leukemia, tumor microenvironment, energy metabolism, TCA cycle, resistance to therapy

O-23: Sf1 is translationally controlled through conserved 5' UTR to regulate differentiation of blood

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RNA splicing consists of removal of introns and ligation of exons to form mature mRNA. This way splicing expands the coding capacity of the genome. This process is carried out by the spliceosome - the biggest ribonucleoprotein complex in the cell. One of the initial steps during splicing is the recognition of the branchpoint within introns. Branchpoint recognition is facilitated by binding of splicing factor 1 (Sf1). Defects in splicing are implicated in many diseases including degeneration and cancer. However, the role of splicing in homeostasis, particularly in stem cell differentiation, remains unclear. Here, I analysed how the levels of Sf1 are regulated during activation of hematopoietic stem cells (HSCs). HSCs are a paradigmatic stem cell system, with important translational value. Our preliminary observations show that Sf1 is dynamically regulated at the protein level during HSCs differentiation. This is in the absence of associated changes in mRNA, suggesting posttranscriptional mechanism of regulation. Strikingly, Sf1 is essential for the loss of stemness. Therefore, our aim was to understand how SF1 is regulated during blood differentiation. To address this question, we analyzed the genomic locus of Sf1, revealing evolutionary conserved and highly structured 398 nucleotides long 5' UTR. To assess the translational control of Sf1, we generated the reporter system with Firefly luciferase (Fluc) controlled by deletion and substitution mutants of murine Sf1 5' UTR. 5' UTR structure predictions were obtained using Vienna RNAFold software, and regions crucial for efficient translation were identified (stem loops 2 and 3). Subsequently, to discover *trans*-acting factors implicated in regulation of Sf1 translation, we performed pulldown experiments with full-length or mutant, translationally deficient variant of Sf1 5' UTR.

Keywords: splicing, RNA structure, hematopoietic stem cells, translational regulation

O-24: The importance of platelet growth factors as markers of survival and severity of COVID-19 in patients undergoing monthly follow-up

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Introduction: COVID-19 is an infectious disease caused by the SARS-CoV-2 virus, which belongs to the family Coronaviridae. In COVID-19-related coagulopathy, platelet activation leads to inflammation and thrombosis. Activated platelets release substances that affect various processes, including inflammation, tissue regeneration, and cancer progression. Research suggests that platelet-derived growth factors like TGF-β, IGF-1, and PDGF-BB may be important markers for COVID-19 severity and survival. Aim of the study: The aim of this study was to analyze the concentrations of TGF-β, IGF-1, and PDGF-BB in patients with COVID-19 compared to the control group. Material and methods: The research group consisted of 50 people (23 men and 27 women) at the time of COVID-19 detection and after 7, 14, and 28 days from the detection of SARS-CoV-2 infection. The control group consisted of 48 healthy volunteers, comprising 25 women and 23 men. The concentrations of TGF-β, IGF-1, and PDGF-BB in plasma were analyzed using ELISA methods. The severity of COVID-19 was evaluated using the MEWS, classifying patients as mild, moderate, or severe. Statistical analysis was performed using the Statistica PL 13 program. Results: A significant relationship was found between TGF-β1, IGF-1, and PDGF-BB levels in both the research group (blood collection I-IV) and the control group (GK). Statistical analysis indicated that TGF-β ($p = 0.013$), PDGF-BB ($p = 0.015$), and IGF-1 ($p = 0.013$) concentrations affect COVID-19 patient survival. The severity of COVID-19 symptoms also significantly influenced IGF-1 ($p = 0.018$), TGF-β ($p = 0.044$), and PDGF-BB ($p = 0.047$) levels. Conclusions: Platelet-derived growth factors may be useful as markers for disease severity and could inform new therapies for COVID-19. Future research should investigate their role in the pathogenesis of COVID-19 to gain a better understanding of the physiological processes during SARS-CoV-2 infection.

Keywords: COVID-19, IGF-1, PDGF-BB, TGF-β

O-25: Development of an Organ-on-a-Chip Microsystem for Endometrium Modeling

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The dynamic development of *Organ-on-a-Chip* technology is opening new possibilities for modelling tissues *in vitro*. My thesis focuses on the development of a microfluidic *Organ-on-a-Chip* microsystem which enables modelling of the endometrium using Human Umbilical Vein Endothelial Cells (HUVEC), endometrial fibroblasts, and endometrial epithelial cells. Developing methods for studying the endometrium is particularly important in treating endometriosis—one of the most common disorders associated with the endometrium. Endometriosis leads to severe consequences, including pain, heavy menstrual bleeding, and most notably, infertility. Currently, no effective therapy or medication for endometriosis exists. Therefore, advanced technologies that facilitate *in vitro* research are important. Given the limited number of scientific publications in this area, this work addresses a research gap.

The aim of my thesis was to determine the optimal geometric parameters for an Organ-on-a-Chip microfluidic device for modelling the endometrium. Measurements included distances between micropillars, lengths of microchannels, and heights of all microstructures. Microstamps were fabricated using two techniques—3D printing and micromilling—to compare the precision of the microstructures' mapping. Additionally, hydrogel properties were evaluated upon introduction into the microchip, with a focus on their structure, density, and tendency to retain air bubbles. Based on these assessments, a single hydrogel—collagen or fibrin—was selected for use.

Following the selection of the hydrogel, cellular tests were conducted to observe self-organization of HUVEC cells without the addition of growth factors. After two days of cultivation, initial cell aggregation was observed, providing hope for achieving a vascular network and functional tissue model in future studies. This Endometrium-on-a-Chip system can serve as a foundation for future research on modelling the endometrium and testing potential drugs for treating endometriosis.

Keywords: Organ-on-a-Chip, Endometrium-on-a-Chip, endometrium, endometriosis, endometrium modeling, HUVEC cells, endometrial fibroblasts, endometrial epithelial cells, collagen, fibrin, micromilling, 3D printin

O-26: Design and Construction of a Magnetic Microreactor for Enzymatic Reactions

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The main goal of this research was to create and manufacture a cost-effective and easily reproducible magnetic microbioreactor designed for biocatalytic applications, operating under a pulsating electromagnetic field. The utilization of open source microcontoler such as ESP32 played a vital role in achieving precise control over the reactor chamber conditions. Both the controller, the chamber, and the housing of the microbioreactor were designed using FreeCAD 1.0.0 and fabricated with 3D printing technology. The main part of the bioreactor consists of a four-chambered thermoblock, maintained at a constant temperature via thermostated water circulation. Each chamber contains two opposing electromagnets capable of generating a pulsating electromagnetic field. The control unit, programmed in the Arduino IDE, includes temperature sensors, a voltage-regulating potentiometer, function keys, and an LCD display for system status monitoring. The functionalities of the microbioreactor were assessed using yeast invertase, immobilized through the magnetic cross-linked enzyme aggregate (mCLEA) approach. This technique involves cross-linking the enzyme in the presence of iron oxide (Fe3O4) nanoparticles, enabling manipulation of the enzyme via magnetic field control. The proposed system facilitates precise modulation of enzymatic activity by calibrating electromagnetic field parameters, unlocking innovative avenues for dynamic management of biocatalytic processes. Additionally, the microbioreactor serves as a versatile platform for both educational and research purposes, making it suitable for academic laboratory use and small-scale biotechnological ventures.

Keywords: Bioreactor, 3D Printing, PLA, mCLEA, PMF (Pulsating Magnetic Field), Arduino

O-27: Research on the use of polymer membranes with magnetic properties for the culturing and maturation of heart cells

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Cardiovascular diseases are the leading cause of death globally, necessitating research to understand their pathophysiology and develop new drugs [1]. The cellular models currently used for this purpose utilize immature heart cells which differ from mature cardiomyocytes in terms of structure and function. An innovative approach in *in vitro* studies is the use of induced pluripotent stem cells - derived cardiomyocytes (iPSC-CMs), although their phenotype is similar to fetal cells [2]. Methods promoting the maturation of cardiomyocytes exist, but achieving their full maturity is not possible yet. Therefore, it is important to conduct further research on cardiomyocytes maturation. The study aimed to develop new polymer substrates with magnetic properties, to facilitate mechanical stimulation of heart cells *in vitro*, enhancing their maturation. Nylon membranes with magnetic nanoparticles capable of deformation using a variable magnetic field were designed and fabricated. Microstructures were created on the membranes to force directed growth of cardiomyocytes. Cell culture and stimulation was possible due to the design and production of a holder for the membrane. A multi-day co-culture of iPSC-CMs with human cardiac fibroblasts (HCFs) was carried out on a polystyrene plate and on the developed membrane. It was found that microstructures on the membranes enable directed growth of cardiomyocytes. Additionally, based on RT-PCR (Reverse Transcription-Polymerase Chain Reaction), an increase in the relative expression of cardiomyocyte maturity genes was found for culture on the membrane with magnetic stimulation. The results indicate that co-culture of HCFs and iPSC-CMs on a membrane with magnetic stimulation affects the increase in cardiomyocyte maturity. The conducted studies may contribute to the development of methods promoting cardiomyocyte maturity, which may ultimately enable the creation of cell models more similar to *in vivo* conditions.

Research financed by the Inicjatywa Doskonałości - Uczelnia Badawcza program as part of the Young PW project 504/04496/1020/45.010026.

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Keywords: cardiovascular diseases, stem cells, cardiomyocyte maturation, polymer membranes, magnetic field stimulation

O-28: Statins Reloaded: Investigating the Oncological Potential of Cardiovascular Drugs

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Drug repurposing is a strategy that involves the application of existing, approved medications for new therapeutic indications. Statins, known as HMG-CoA reductase inhibitors, are widely used for lowering cholesterol and protecting the cardiovascular system. Increasing evidence suggests that statins may also hold promise in cancer prevention and treatment. The mechanisms of statins extend beyond cholesterol biosynthesis inhibition include pleiotropic effects such as modulation of angiogenesis, apoptosis, autophagy, metastasis, and the tumor microenvironment. Moreover, recent clinical studies suggest the potential for combining statins with conventional anticancer drugs, opening avenues for synergistic combination therapies.

The aim of this study was to evaluate the anticancer potential of repurposed drugs - statins - and to assess their safety in the model organism *Caenorhabditis elegans*. The cytotoxicity of selected statins was assessed using the Neutral Red (NR) assay against glioblastoma (U-118 MG), tongue squamous cell carcinoma (SCC-15) and non-cancerous keratinocytes (HaCaT) at concentrations ranging from 3.125 to 100 M. *C. elegans* (Bristol N2 strain) were synchronised by bleaching and incubated at 20°C for 21 days with statins at concentrations between 12.5 and 200 M. Daily observations - morphology, behaviour and survival were monitored using a Delta Optical IB-100 inverted microscope. Data were analysed using Statistica 13. Among the statins tested, atorvastatin, simvastatin and lovastatin showed the strongest anti-cancer effects, significantly reducing the viability (by 40-80%) of U-118 MG and SCC-15 cells at concentrations between 25-100 M. In terms of safety, pravastatin caused the lowest decrease in survival of *C. elegans* after 21 days of exposure (approximately 40%). However, atorvastatin at the highest concentration (200 M) reduced survival to 65%. All statins tested induced behavioural changes and morphological abnormalities (degeneration of internal organs and cuticle).

Preliminary results suggest that certain statins, particularly atorvastatin, may be promising candidates for repurposing in the treatment of glioblastoma. However, the safety of using doses above approved therapeutic levels requires further investigation in higher organisms.

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Keywords: glycidylated PAMAM dendrimers, foams, polymer matrix, doxorubicin, SCC-15, HaCaT cells

O-29: Anisakis simplex extracellular vesicles as modulators of oxidative stress in host cells

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Extracellular vesicles (EVs) are lipid membrane-bound structures that facilitate communication between cells and play a crucial role in parasite-host interactions. While the host's immune response to parasitic infections is primarily regulated by Th2 lymphocytes, oxidative stress is also a key factor in modulating host-pathogen interactions. However, the role of *Anisakis simplex*-derived EVs in regulating oxidative stress in the host remains unexplored. This study aimed to investigate the modulation of oxidative stress by *A. simplex*-derived EVs (Anis-EVs). Caco-2 cells were treated with two concentrations of Anis-EVs (Low and High), and oxidative stress markers, including malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) activity, were measured. MDA levels, a marker of lipid peroxidation, significantly decreased in EV-treated cells. The MDA concentration in the Low EVs group was 24.32 µM, and in the High EVs group, it was 8.96 µM, compared to 43.49 µM in the control. SOD activity increased in EV-treated cells, with 109.25% inhibition in the control, 86.60% inhibition in the Low EVs group, and 66.37% inhibition in the High EVs group. GSH levels decreased, with 4.99 mmol/L in the control group, while cells treated with Low and High EVs concentrations exhibited 4.32 mmol/L and 4.41 mmol/L, respectively. Statistical analysis revealed a significant reduction in GSH levels between the control and the Low EVs group, but no significant difference between the control and the High EVs group, or between the two EV-treated groups. The findings indicate that Anis-EVs modulate oxidative stress mechanisms in the host by decreasing lipid peroxidation and altering antioxidant enzyme activity. While EVs significantly affected MDA levels and SOD activity, their impact on GSH content was limited. This study provides new insights into parasite-host interactions and highlights the need for further research on oxidative stress during parasitic infections.

Keywords: extracellular vesicles, oxidative stress, Anisakis simplex

O-30: The impact of *Toxocara canis* antigens on the cytokine response of lung epithelial cells and macrophages in an in vitro co-culture model

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Toxocara canis is a common parasite found in the intestinal tract of dogs. This parasite can be transmitted through contaminated soil, vegetables, meat, etc. It has zoonotic potential and causes a disease in humans known as toxocariasis. This helminth infection can affect the respiratory system. It is possible due to the release of *Toxocara* excretory-secretory antigens (TES), which can modulate the host immune response. Recent studies suggest a potential link between toxocariasis and asthma. Since the pathogenesis of asthma remains unclear, research into the mechanisms of toxocariasis may provide valuable insights. This study aimed to develop an *in vitro* model to analyze the effects of parasitic antigens on the function of alveolar and bronchial epithelial cells in association with macrophages to illustrate the interactions between epithelial and immune cells. As part of the research, two co-culture models were established: a direct co-culture and a co-culture with inserts, in which the cells were physically separated. Cells were stimulated with TES antigens or *T. canis* larvae. Cytokine concentrations in the culture media were measured using the ELISA method. Data obtained from cell co-culture models show that the presence of both cell types, epithelial cells, and macrophages induces the production of proinflammatory cytokines (IL-6, IL-1β, TNF-α, CXCL8) in co-culture conditions. Moreover, TES molecules further upregulate the secretion of these cytokines. The influence of *T. canis* larvae was lower compared to TES. This will enable an initial in vitro investigation of immune processes involved in host-parasite interactions within the lungs.

This study was supported by a research grant no. 2020/39/B/NZ6/02176 from the Polish National Science Center.

Keywords: Toxocara canis, cytokines, co-culture, lung

O-31: Analysis of the expression and activity of proteolytic enzymes of the pitcher plant (*Nepenthes x ventrata*) during nitric oxide-supplemented digestion

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The pitcher plant (*Nepenthes x ventrata*) is a carnivorous plant that uses traps to capture insects for nutrients (mainly nitrogen and phosphorus). Inside the pitchers is a digestive fluid containing hydrolytic enzymes such as proteases, chitinases and esterases. The proteases from digestive fluid, due to its properties, can be widely used in medicine, agriculture and other industries. Pitcher plant has unique proteases named Nepenthesins. These enzymes are insensitive to the temperature and pH of the environment and have the capacity for autocatalysis. Proteases catalyze the breakdown of peptide bonds in proteins. The purpose of this study is to determine the influence of nitric oxide (NO) on the activity of proteases. Studies on the effect of NO were conducted on traps 1,2 and 4 days after protein and NO supplementation. Protease activity was determined spectrophotometrically using azocasein as a substrate. Moreover activity of proteases was determined by zymography. The influence of NO on ubiquitin-proteasome system was examined using the western blotting technique. Expression of genes coding Nepenthesins was studied using qRT-PCR. The highest protease activity was found in the digestive fluid from the traps after two days of digestion. A significant increase in protease activity was observed on the first day after feeding in the NO-supplemented sample, compared to the sample fed with protein.

Keywords: Proteases, Pitcher plant, Nitric oxide

O-32: Apis mellifera royal jelly-derived extracellular vesicles as a potential anti-aging tool.

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Equine metabolic syndrome (EMS) is characterized by chronic low-grade inflammation and heightened cellular senescence, compromising the regenerative potential of adipose-derived stem cells (EqASCs). With growing interest in harnessing natural bioactive compounds for anti-aging interventions, this study investigates the regenerative potential of extracellular vesicles (EVs) derived from *Apis mellifera* royal jelly (RJ-EVs) as a novel therapeutic strategy to alleviate cellular dysfunction in EMS-affected EqASCs. EqASCs isolated from EMS horses were treated with RJ-EVs, and their impact on senescence-associated markers was meticulously evaluated. RJ-EV administration led to a profound modulation of senescence pathways, notably reducing H_2AX expression—indicative of diminished DNA damage accumulation—while also orchestrating apoptotic signalling to favour cellular equilibrium. These effects suggest a potent role of RJ-EVs in rejuvenating metabolically compromised stem cells by restoring homeostasis and enhancing cellular resilience. These findings underscore the promising potential of RJ-EVs as an innovative anti-aging intervention, offering a compelling avenue for regenerative therapies in both veterinary and biomedical applications. Further exploration into their molecular mechanisms could pave the way for transformative strategies in stem cell-based rejuvenation and metabolic disorder management.

Keywords: Royal jelly, extracellular vesicles, stem cells, equine metabolic syndrome, senescence, regenerative therapy

P-1: Proteolytic processing of adhesion GPCR

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Adhesion G-protein coupled receptors (aGPCR) are key to regulating many processes, such as cell migration and adhesion. aGPCRs are membrane proteins that undergo autoproteolysis in the endoplasmic reticulum during maturation. This process occurs within the GPCR autoproteolysis-inducing (GAIN) domain and leads to the formation of two fragments: an N-terminal fragment (NTF) and a C-terminal fragment (CTF) that penetrates the cell membrane seven times. The NTF and CTF fragments remain linked together by noncovalent bonds. When NTF is released into the extracellular environment, for example by interacting with extracellular matrix proteins leading to the disruption of noncovalent bonds between NTF and CTF, the conformation of CTF changes and the receptor is activated. It is postulated that extracellular sheddases may also participate in the release of NTF. In order to verify the role of sheddases in the release of NTFs of various aGPCR receptors, it was decided to create cell lines with inducible overexpression of sheddases from the ADAM family. For this purpose, the Sleeping Beauty method was used. The effect of sheddases on the release of aGPCR NTFs was studied using flow cytometry and western blot analysis, analyzing the correlation between NTF presented on the surface of the cell membrane, NTF released into the culture medium and CTF remaining in the cell membrane. The obtained results indicate that sheddases stimulate the release of NTFs from the cell surface. This phenomenon may be significant in the development of therapies against diseases in which abnormal functioning of aGPCR receptors is observed.

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Keywords: aGPCR, sheddases, ADAM, proteolysis

P-2: The potential role of arachidonic acid derivatives as biomarkers for assessing prognosis and mortality in COVID-19 patients during a month observation

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Background: COVID-19, caused by the SARS-CoV-2 virus, became a global pandemic in 2019, leading to long-term complications for many. Unsaturated fatty acids, like arachidonic acid (AA) and its metabolites (prostaglandins and leukotrienes), can inactivate viruses and regulate inflammation. Exploring the relationship between COVID-19 and AA derivatives may help understand its long-term effects.

Objective: The objective of this study is to investigate the effects of arachidonic acid derivatives (5-HETE,15-HETE, thromboxane-TXB2, and ALOX-5 on the progression and mortality associated with COVID-19 in patients

Materials and Methods: The study involved 53 patients (25 men and 28 women) assessed for COVID-19 detection. They were followed up at 7, 14, and 28 days (I-IV groups). A control group (CG) of 48 healthy volunteers (25 women and 23 men) was used. Blood serum was analyzed using ELISA methods. The severity of COVID-19 was evaluated using the MEWS, classifying patients as mild, moderate, or severe. Statistical analysis was performed using Statistica PL 13 Trial software.

Results: The levels of arachidonic acid derivatives varied with disease duration. Group III had the highest 5-HETE levels, while the control group had the lowest ($p<0.001$). Conversely, the control group showed the highest concentration of 15-HETE ($p=0.003$) and TXB2 ($p<0.001$). Patients who died from COVID-19 had significantly higher levels of 5-HETE ($p<0.001$) and TXB2 ($p=0.005$) than survivors. The severity of COVID-19 also affected 15-HETE levels ($p=0.001$).

Conclusions: Analyzing arachidonic acid derivatives is important for assessing survival and disease progression in COVID-19. AA and its metabolites may have antiviral properties, and deficiencies could increase susceptibility to SARS-CoV-2. AA derivatives could serve as biomarkers of disease severity, warranting further research on their therapeutic potential.

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Keywords: COVID-19, 5-HETE, 15-HETE, thromboxane-TXB2, ALOX-5

P-3: The Role of Nuclear Factors in Programmed Cell Death Induction in CRISPR/Cas9-Modified Cells

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The pulmonary epithelium serves as the first line of defense in the respiratory system against environmental stressors. Its ability to respond to oxidative stress is crucial for maintaining cellular homeostasis. Under conditions of increased reactive oxygen species (ROS) production, protective mechanisms such as the thioredoxin pathway (TRX/TXNRD1) and the nuclear factor NRF2 are activated. Disruptions in these signaling pathways can lead to heightened susceptibility to ferroptosis—a form of programmed cell death triggered by lipid peroxide accumulation. This study investigates the impact of thioredoxin reductase-1 (TXNRD1) inhibition on NRF2 activation and ferroptosis mechanisms in human bronchial epithelial cells (BEAS-2B) genetically modified using CRISPR/Cas9 technology. Three cell lines were utilized: wild-type (WT), GPX4 knockout (KO), and a positive control (PC). Cells were exposed to erastin, an inhibitor of the cystine transporter (XC^-) and a known ferroptosis inducer. The expression levels of NRF2, TRX, and TXNRD1 were assessed using RT-qPCR. The results revealed that GPX4 deletion led to a significant reduction in TRX and TXNRD1 expression, impairing the cells' ability to activate compensatory mechanisms against oxidative stress. At the same time, KO cells exhibited increased NRF2 expression in response to higher erastin doses, suggesting the activation of alternative protective pathways. Correlation analysis confirmed that the TRX/TXNRD1 and NRF2 pathways co-regulate ferroptosis, but their effectiveness depends on the presence of GPX4. These findings highlight the critical role of NRF2 in oxidative stress response when the thioredoxin system is compromised. NRF2 activation may serve as a compensatory mechanism in GPX4-deficient conditions. The study underscores the interplay between antioxidant pathways in ferroptosis regulation and suggests new directions for therapeutic strategies in oxidative stress-related diseases, such as cancer and neurodegenerative disorders.

Keywords: Ferroptosis, oxidative stress, NRF2, thioredoxin pathway, TXNRD1, GPX4 knockout, CRISPR/Cas9

P-4: Optimizing whole-grain bread recipe with microencapsulated polyphenols for functional benefits

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In response to the growing consumer demand for functional foods, this study aimed to develop an innovative bread formulation enriched with microencapsulated polyphenols, oat β-glucan concentrate, and sour fermented beetroot juice. The objective was to enhance the bread's nutritional profile and health benefits while maintaining sensory appeal. Microencapsulation was used to protect polyphenols from thermal degradation, preserving their bioactivity. The effects of functional ingredients on physicochemical properties, including dough viscosity, hardness, porosity, bioactive compound content, color, and volatile compounds, were analyzed. Sensory acceptability was also assessed. Using response surface methodology, the formulation was optimized to maximize polyphenol and β-glucan content. The optimized composition included 4.60% sour fermented beetroot juice, 6.29% β-glucan concentrate, and 2.77% microencapsulated polyphenols. The final bread exhibited high antioxidant activity and consumer acceptability, with functional ingredients improving texture and sensory characteristics. The findings highlight the potential of this bread as a functional alternative to traditional bakery products, offering health benefits associated with polyphenols and β-glucan, such as anti-inflammatory and antioxidant properties. Given the increasing market interest in functional foods, natural ingredients, and health-conscious eating, this study underscores the role of innovative formulations in promoting a balanced diet and encouraging healthier consumer choices.

Keywords: nutritional enhancement, β-glucan concentrate, sour fermeneted beetroot juice, black carrot microencapsulated phenols, response surface methodology optimization

P-5: The antioxidant and anti-aging activity of *Helichrysum arenarium* callus tissue

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Helichrysum arenarium (Asteraceae), is also known as immortelle. Due to its biological properties it has been used in traditional medicine for centuries for antiseptic, anti-inflammatory, hepatoprotective and relaxant properties [1]. The aim of the study was to evaluate the phytochemical and biological properties of H. arenarium callus tissue which have not been previously explored. The callus was cultivated on solid Murashige and Skoog (MS, 1962) [2] medium with BAP 1 and 2,4-D 1 mg/L. Four-week-old callus was collected, lyophilized and subjected to the total phenolic content (TPC), total flavonoid content (TFC), antioxidant properties (DPPH and FRAP methods) and anti-collagenase activity investigations [3]. The extracts were prepared with absolute or 70% methanol (MeOH). The highest TPC (56 GA mg/g of extract) and TFC (1.4 QE mg/g of extract) was detected in absolute MeOH extract. The same extract exhibited the highest antioxidant activity determined by DPPH (58.87 %) and FRAP (24.85 TE mg/g of extract). All extracts were more potent in collagenase inhibition than standard compound i.e. epigallocatechin gallusan, with the highest activity demonstrated by 70% MeOH extract - up to 29 %. The results of this study suggest that the solvent type used determined the TPC and TFC in extracts. It also corresponded to the highest antioxidant power of these extracts. Nevertheless, further investigations should be carried out to determine which group of secondary metabolites is responsible for anti-collagenase activity of tested extracts.

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Keywords: Callus tissue, in vitro, anti-collagenase, Asteraceae

P-6: Effect of magnetic fields on L929 murine fibroblasts

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Background. Previous research showed that magnetic field (MF) exerts an inhibitory effect on various parameters of microorganisms, including growth rate, viability, and metabolic activity. It was demonstrated that MF enhances the efficacy of antimicrobial agents against *Staphylococcus aureus*. These findings suggest that MF could be a promising therapeutic tool for treating infections, especially those caused by antibiotic-resistant bacteria. However, to fully explore the therapeutic potential of MF, it is essential to understand its impact on both microorganisms and eukaryotic cells, such as fibroblasts. Studying the impact of MF on skin cells will provide insights into the safety and potential applications of MF-based therapies, particularly in treating infected wounds and biofilm-associated infections.

Aim. This study aimed to explore the impact of selected parameters of MF on L929 murine fibroblasts.

Methods. L929 murine fibroblasts were cultured (0.3×10^6 cells/dish) in DMEM for 24 h (37^oC, 5% CO_2) for cell adhesion. After incubation, DMEM was aspirated, cells washed, and fresh DMEM added. Cells were exposed to MF for 1 h using sine and triangle waveforms at 5, 50, and 2000 Hz. Control cells were incubated without MF. After exposure, cells were washed, detached, centrifuged, and resuspended in DMEM. Three assays evaluated cell viability (LIVE/DEAD assay), metabolic activity (AlamarBlue assay) and morphological changes (via an inverted microscope).

Results. Results indicated that frequency and waveform affected cell parameters. Higher frequency was associated with more dead cells and a decreased number of live cells. The sine wave resulted in more live cells than the triangle wave. Metabolic activity was inhibited at the highest frequency, while stimulated at the lowest. The triangle wave had a stronger inhibitory effect compared to sine wave. At the highest frequency, cells showed reduced spreading and increased rounding, regardless of waveform. Other frequencies did not elicit visible morphological changes.

Keywords: frequency, magnetic field, metabolic activity, morphology, murine fibroblasts, viability, waveform

P-7: The influence of selected methylxanthines on the interactions with pulmonary surfactants at the water-air interface

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Chronic Obstructive Pulmonary Disease (COPD) is a common respiratory disorder characterized by progressive airflow limitation and lung tissue destruction. The main causes include chronic inflammation caused by prolonged exposure to harmful particles or gases, including cigarette smoke. Symptoms include coughing, shortness of breath, and mucus production, and their severity can lead to respiratory failure. According to WHO Europe data, COPD affects 5-10% of adults over 40 years old, which translates to approximately 23 million people in Europe. Projections indicate that COPD will become the third leading cause of death worldwide by 2030. [1,2]

The study examined the effect of selected drugs from the methylxanthine group, including theophylline derivatives, on model pulmonary surfactants with a composition similar to that of real pulmonary surfactants. The aim of the work was to preliminarily verify whether it is possible to apply methylxanthines via inhalation. The lipid/drug systems analyzed consisted of lipids such as DPPC and DPPG and their mixtures DPPC:DPPG (in a molar ratio of 8:2), and the drugs were theophylline and theophyllinic acid. The research involved preparing and characterizing model pulmonary surfactants using the Langmuir method and analyzing changes in the morphology of layers at the phase boundary using Brewster angle microscopy (BAM). The results showed that theophylline strongly interacts with DPPC, changing the shape of the π-A isotherm and reducing the compressibility coefficient, while theophyllinic acid significantly affects DPPG, causing a phase transition and reorganization of molecules. Meanwhile, the isotherm of the DPPC:DPPG (8:2) mixture for theophyllinic acid resembles the DPPG/theophyllinic acid system, and the isotherm of the DPPC:DPPG (8:2) mixture for theophylline is similar to the DPPC/theophylline system. BAM images confirmed these observations, showing analogies in layer morphology. The conducted studies not only reveal a possible mechanism of interaction between theophyllin

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Keywords: Lung surfactants, phospholipid monolayers, methylxanthines, chronic obstructive pulmonary disease (COPD), Langmuir method, Brewster Angle Microscopy (BAM)

P-8: On the role of intercalating residues in restriction associated dsDNA endonucleases

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Intercalation of one or more protein amino acids into dsDNA is a frequent feature in protein-nucleic acid complexes. It is often associated with detection and repair of damaged DNA, enzymatic DNA base modification, modification readout, or with long-range DNA structure modulation by kinks and bends. In the context of restriction modification (RM), it is frequently associated with indirect sequence recognition and detection of methylated bases. With the ThaI and VcaM4I dsDNA complexes, we have selected well-characterized representative examples of the key intercalation types in RM systems. In the ThaI case, we show that alterations to the intercalating residue(s) destabilize the specific complex and reduce single turnover kinetics. Surprisingly, however, mutants remain capable of specific DNA cleavage, and are even able to catalyse the cleavage faster than the wild-type enzyme in multiple turnover conditions. This unexpected tolerance towards amino acid substitutions is explained by crystal structures that show solvent molecules making up for the missing part of the intercalating amino acid side chain. In the case of VcaM4I, we find that removal of the intercalating side chain abolishes the discrimination between methylated and unmodified DNA, but not the catalytic proficiency of the enzyme.

Keywords: DNA, restriction endonuclease, methylation

P-9: Characteristics of oils extracted from sunflower seeds roasted with thyme

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Sunflower seeds are a popular snack and a valuable addition to various bars and muesli-type breakfast mixes. They are a rich source of fat, vegetable protein, fibre and vitamins. They have a beneficial effect on health, supporting the nervous and circulatory. Their roasting gives them a more crumbly and tender texture and a characteristic taste and smell, while denaturing the proteins increases their digestibility. Sunflower seeds are also used to produce sunflower oil. In addition, it is also used in cosmetics and as an ingredient in pharmaceutical products such as ointments and emulsions. This study aimed to evaluate selected quality parameters of sunflower seeds and the oils obtained after roasting them with dried thyme (*Thymus vulgaris* L.) at a weight ratio of 8:1 (w/w) and 160°C for 10 and 30 minutes. Sunflower seeds, unroasted and roasted without and with thyme, were characterized by moisture content, water activity and colour. In turn, for oils obtained from them, the colour, water activity, acid value, peroxide value and fatty acid composition were determined. The study showed that the moisture content and water activity of sunflower seeds decreased after roasting compared to unroasted seeds, with the values obtained being lowest when roasting was done for 30 minutes in the presence of thyme. Roasting sunflower seeds also gave lower values for colour parameters such as L*, a* and b*, except for the sample roasted with thyme for 30 minutes. The oils obtained from roasted sunflower seeds were also slightly darker than those not roasted. The presence of thyme during the roasting of sunflower seeds did not significantly affect the fatty acid profile and water activity of the oils obtained but affected the acid and peroxide values. Oil samples obtained from sunflower seeds roasted with thyme showed slightly lower acid values than their counterparts roasted without this spice. In turn, the peroxide values were significantly lower when the roasting was carried out with thyme addition.

Keywords: sunflower oil, roasting, thyme, quality parameters

P-10: Novel droplet-based microcapsule system for microbial growth

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Introduction. Droplet-based microcapsule systems are an emerging tool with significant potential for microbial and eukaryotic cell culture applications. We present an aqueous two-phase system (ATPS) with a core-shell structure designed to create controlled microenvironments for microbial growth. Using a flow-focusing microfluidic device, we encapsulated bacterial cultures within these microcapsules to enable single-cell studies.

Experimental. As a model organism, we encapsulated fluorescent *E. coli* within V = 170 pL microcapsules featuring a dextran-based core and a shell composed of gelatin methacryloyl (GelMA) or poly(ethylene glycol) diacrylate (PEGDA), combined with the LAP (lithium phenyl-2,4,6-trimethylbenzoylphosphinate) photoinitiator. The shell-core structure formed through liquid-liquid phase separation (LLPS), after which the GelMA/PEGDA shell was photopolymerized, creating a semi-permeable barrier that retained bacterial cells while permitting nutrient and metabolite diffusion. The microcapsules were transitioned from an oil-water emulsion to a fully aqueous environment before being cultured in LB broth at 37°C.

Results. We successfully established a reproducible microfluidic workflow for monodisperse microcapsule generation, achieving stable microbial encapsulation and encapsulated culture. However, optimization is required to enhance encapsulation efficiency and cell viability. Ongoing work aims to refine the system for improved microbial growth and to explore its application in bacterial consortia studies. The combination of microcapsules with single-cell methods holds promise for high-throughput bacterial screening and the cultivation of previously unculturable strains.

Conclusion. Our microfluidic-based microcapsule system provides a streamlined and adaptable platform for single-cell microbial studies. This approach has broad potential applications in microbiology, particularly as single-cell research continues to expand in scope and significance.

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[1] Rathore S. et al. (2013) Microencapsulation of microbial cells. Journal of Food Engineering 116: 369-381.

Keywords: microfluidic, microcapsule, microbiology, microbial consortia, enviromental screening,

P-11: Investigating the oligomerization of IFIT1 protein involved in the cellular immune response

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The innate immune response is a series of pathways that lead to the body's defence against pathogens. Its main components are various physical and chemical barriers, but also a number of cells and proteins that recognize and eliminate foreign particles. Among them, proteins that recognize the genetic material of the virus, including proteins of the IFIT family, play an important role in the defence against the development of viral infection. The element of interest in this study was the IFIT1 protein, which recognizes viral RNA in the form of ppp-RNA or with a cap 0 structure. Preliminary studies, suggest that the IFIT1 protein is capable of forming advanced oligomeric structures. The present research was designed to investigate this process and characterize the aggregates formed. For this purpose, the native IFIT1 protein and its mutants, in which the domain responsible for the homodimerization process was modified, were expressed. The aggregates were obtained by incubating the proteins at 37°C, and their stability was tested using proteinase K digestion and observing the degradation products. For preliminary characterization of the formed structures, the protein aggregates were subjected to observation by differential light scattering (DLS). The obtained IFIT1 proteins produced advanced oligomeric forms, which differed in both structure and stability. The most stable forms were obtained for the native IFIT1 protein, and the least stable for the double mutant protein. The formation of oligomeric forms was also confirmed by DLS experiments. Study of the oligomerization of the IFIT1 protein and the role of the structures that are formed in this process opens up a new, previously unknown area in the broader immune response process.

Keywords: immune response, IFIT proteins, IFIT1, viral infections, protein oligomerization

P-12: Natural products as bioinsecticides — effects of solamargine on the immune-related mechanisms in *Tenebrio molitor* L.

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Insects are the largest group of animals in the world that impact on human welfare and the environment, including agricultural production. They play an important role as pollinators but on the other hand they can also contribute to significant losses in agriculture. Many synthetic insecticides are characterized by a low specificity towards certain insects species. Moreover, an increasing number of insect species are developing resistance to currently used insecticides. Due to this, the knowledge about potential alternatives for synthetic insecticides has significantly increased in recent years. One of the possibilities is the use of plant extracts and their glycoalkaloids (GA). Our recent research showed, that *Solanum nigrum* fruit extract (EXT) can affect the immune system activity of mealworm beetle, *Tenebrio molitor* L., which is a serious pest of stored crops (Urbański et al., 2023). For this reason, our next step was to analyze one of the main components of the EXT. In this study, we analyzed the influence of solamargine on selected immune-related parameters. It is one of the main GA of the EXT. Solamargine (0,1% and 0,01%) was injected into 7-8-day-old male *Tenebrio molitor*. We analyzed the effect of this GA on survival of beetles, phenoloxidase acitivity and the expression level of the immune-related genes such as receptor Toll, Relish transcription factor and selected antimicrobial peptides. The obtained results showed, that solamargine affects the immune system activity, but this effects are different from those obtained in our previous studies. This may be related to the synergistic action of various EXT components, such us GA. It is a crucial step toward a complete understanding of the EXT and GA mechanisms of action on insects' physiology and its potential usage as bioinsecticides.

Research funded by Excellence Initiative — Research University project (118/34/UAM/0024).

[1] Urbański A. et al. (2023) Solanum nigrum Fruit Extract Modulates Immune System Activity of Mealworm Beetle, Tenebrio molitor L.. Toxins 15: 68.

Keywords: bioinsecticides, solamargine, insect immune system, Tenebrio molitor

P-13: *Porphyromonas gingivalis* and *Bacteroides fragilis* HmuS — a new family of proteins with dechelatase activity

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Porphyromonas gingivalis and *Bacteroides fragilis,* both members of the Bacteroidota phylum, play a significant role in the pathogenesis of chronic human diseases. *P. gingivalis* is associated with dysbiosis in the oral microbiome leading to the initiation and progression of periodontitis, while *B. fragilis* is associated with colon inflammatory diseases and after spreading with intra-abdominal infections, endocarditis, and often sepsis. Together, they exacerbate chronic inflammation, increasing the risk of systemic diseases such as colorectal cancer, pancreatic cancer, Alzheimer's disease, and atherosclerosis. *P. gingivalis* and *B. fragilis* lack functional heme biosynthesis pathways and rely on heme as their primary source of iron and protoporphyrin IX. To survive, they must acquire heme from human hemoproteins. For this, they utilize the Hmu system, which consists of a hemophore-like protein (HmuY), an outer membrane transporter (HmuR), and other proteins with functions yet to be defined. Among them is the HmuS protein, a putative chelatase with homology to the CobN protein. The mechanism of iron extraction from heme in anaerobic *P. gingivalis* and *B. fragilis* remains unknown, and it is hypothesized that HmuS functions as a dechelatase involved in this process. Therefore, using both theoretical and experimental approaches this study aimed to analyze the function of the HmuS protein from *P. gingivalis* ($HmuS^{Pg}$) and *B. fragilis* ($HmuS^{Bf}$) in heme uptake and metabolism. Heme-binding simulations for both proteins indicate a high degree of similarity in their binding pockets and key residues involved in heme-iron coordination. Using spectroscopic methods, we have demonstrated that both proteins bind heme in oxidized and reduced conditions, and under reducing conditions, they can remove iron from heme. These results show that $HmuS^{Pg}$ and $HmuS^{Bf}$ are involved in iron acquisition, highlighting their critical role in the survival of bacteria in hostile human environments.

This study was supported by grant no. 2021/41/B/NZ6/00702 received from the National Science Center (NCN) Cracow, Poland.

Keywords: Porphyromonas gingivalis, Bacteroides fragilis, dechelatase, chronic human diseases, heme uptake,

P-14: Green nanoparticle synthesis in the application of non-bacterial mastitis in cattle

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Non-bacterial pathogens like yeasts and algae present challenges in medical microbiology due to their resistance to conventional treatments. This study assessed green-synthesized silver nanoparticles (AgNPs) as an alternative antimicrobial strategy. AgNPs were synthesized using coffee extract and tested against yeast species (*Candida albicans, Pichia fermentans, Pichia kudriavzevii, Wickerhamomyces anomalus, Wickerhamiella pararugosa*) and the algal strain *Prototheca bovis*. Antimicrobial activity was evaluated through minimum inhibitory (MIC) and bactericidal (MBC) concentrations, as well as viability, biofilm formation, and invasion assays. AgNPs demonstrated broad-spectrum inhibitory effects, with *Prototheca bovis* showing moderate sensitivity compared to highly susceptible yeasts like *C. albicans* and *P. fermentans*. Biofilm formation and invasiveness were significantly reduced across all tested strains, with higher AgNP concentrations leading to diminished colony density and biofilm biomass. These findings highlight the potential of green-synthesized AgNPs as an effective antimicrobial agent against resistant non-bacterial pathogens. Their ability to disrupt biofilm formation and invasiveness, particularly in *Prototheca bovis*, suggests their promise as an alternative to conventional treatments. Further research is needed to optimize AgNP applications in clinical and environmental microbiology.

Keywords: Yeasts; Prototheca spp.; mastitis; green synthesis; silver nanoparticles

P-15: Advancing 3D Printing in Dentistry: Study on Printing Accuracy and Photopolymerization Shrinkage

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High resolution printouts with low shrinkage are essential in the dental industry, where precision and dimensional fidelity is needed. Traditionally, dental restorations have been created by dental technician using crafting techniques. However, 3D printing has been transforming modern dentistry, enabling automatic production of precise dental models, crowns, bridges and other dental products. 3D VAT photopolymerization is commonly used due to its ability to produce complex and reliable structures from UV-curable resins. Still, this method faces several challenges like print accuracy and polymerization shrinkage. Factors such as printer type, layer exposure time and layer height all significantly influence the achieved printout. Improper parameters can lead to dimensional inaccuracies, affecting the applicability of printed models, thus lowering their use and value in the dental industry.

Another difficulty of 3D VAT printing is the use of 405 nm light source, which is widely used nowadays in commercially available printers. As opposed to lower wavelengths used in the past, this one is in the visible light spectrum, offering lower cost and higher energy efficiency. Even so, 405 nm light poses various challenges like lower light penetration, requiring careful optimization of printing parameters.

This work evaluated and compared various printing configurations and technologies, offering practical insight into optimizing 3D printing for dental applications. The findings permitted us to conclude that printer type selection as well as fine-tuning the printing process significantly affected the quality of the achieved printed object. This research provides guidelines for optimizing 3D VAT printing in the dental industry.

LIDER Program, grant number: LIDER13/0156/2022.

Keywords: 3D printing, resolution, shrinkage, photopolymerization, polymer

P-16: Antibacterial and anticancer properties of kombucha enriched with onion juice or dandelion root juice

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Natural remedies and healthy foods are gaining popularity due to increasing interest in alternative health practices. Kombucha, a fermented tea originating from China, has found its way to many tables due to its health benefits, including improved digestion. Studies suggest that kombucha may help prevent the growth and spread of cancer cells, potentially due to its high concentration of tea polyphenols. The anticancer activity of tea polyphenols is not well understood, but it is believed they may block gene mutations and inhibit the growth of cancer cells. Onion juice and dandelion root juice are plant-based products which thanks to their medical virtues are used in a plethora of indigenous cultures. The juices exhibit both antioxidant and anti-inflammatory properties, which may contribute to their anticancer effects. The aim of this study was to develop a method for preparing kombucha with onion and dandelion root extracts, which would exhibit antibacterial properties against pathogenic bacteria, as well as anticancer properties against liver cancer cells. The methodology involved preparing kombucha from black tea and a purchased SCOBY culture, along with preparing the onion and dandelion root juices. One week after starting the kombucha fermentation, the plant extracts were added to initiate a secondary fermentation. The resulting preparations were evaluated for their antibacterial and anticancer properties. The study allowed for the assessment of kombucha enriched with plant juices as a potential dietary component with therapeutic properties. The results showed that dandelion root extract and kombucha enriched with this extract demonstrated superior anticancer and antibacterial properties, thus further analyses are focused on this version.

Keywords: kombucha, onion juice, dandelion root juice, cancer cells, bacterial strains, HepG2, CaCo2

P-17: Smart sensors for smarter healing: aptamer layers and printed electronics in wound care

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Accurate assessment of the wound during the healing process is essential for appropriate selection of future treatment and care methods. Traditional approaches of wound monitoring have limited effectiveness and are often based on outdated methods or physicians' subjective opinions. In recent years, diagnostic tools based on aptasensors emerged as potential solutions for non-invasive and precise controlling of the healing process. The aim of our research is to develop receptor layers composed of DNA aptamer sequences for electrochemical detection of inflammatory proteins (such as Interleukin 6, TNF-α) which act as biomarkers of the wound healing process. We focused on optimizing the aptamer tethering method on transducer surface by selecting the appropriate incubation times of receptor layer components and their concentration. In our research we investigated the efficiency of receptor layer immobilization on different types of carbon surfaces such as glassy carbon or edge plane pyrolytic graphite (EPPG) electrodes. In addition, we are currently developing a biosensing system using screen-printed electrodes, which could be an ideal tool for non-invasive wound healing monitoring. Moreover, the studies also included the choice of redox indicator to evidence the binding between aptamer-based layer and target analyte, as well as actions aimed on minimization of the time required for sensor preparation and its operation.

Research was funded by the Warsaw University of Technology within the Excellence Initiative: Research University (IDUB) programme.

Keywords: aptamer, biosensors, wound healing, screen-printed electrodes

P-18: Optimization of Biomass Production Parameters for Probiotic Microorganisms *Lactobacillus apis* and *Bombella apis*

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The biomass production of probiotic microorganisms plays a key role in the development of functional products. Its technological features determine the quality and effectiveness of the resulting biomass, influencing its application in medicine and the food industry. This study focuses on two promising probiotic strains: *Bombella apis* — a gram-negative, strictly aerobic, rod-shaped, non-motile bacterium with high probiotic potential for honey bees - and *Lactobacillus apis* - a gram-positive, anaerobic, rod-shaped bacterium known for its lactic acid fermentation capabilities. Both belong to genera of interest for probiotic and industrial biotechnology. The aim of the study was to optimize biomass production by evaluating their growth performance in different culture media: MRS, glucose-peptone, and Mannitol agar. The reactivation of probiotic cultures was performed at 37°C for 48 hours under controlled thermostatic conditions. Biomass accumulation proceeded over a 72-hour period with periodic medium replenishment to support active growth. The most robust proliferation was observed in glucose-peptone and MRS media, whereas Mannitol agar did not support visible growth. Biomass concentration was assessed via spectrophotometric measurement of optical density at 600 nm (OD_{600}), with corresponding CFU-equivalent estimates as follows: *Lactobacillus apis* — 11.4 (MRS) and 6.6 (glucose-peptone); *Bombella apis* — 3.2 (MRS) and 4.6 (glucose-peptone). Final biomass yields after 72 hours of cultivation were 2. 904 g for *L. apis* and 2.62 g for *B. apis*. The findings demonstrate that the choice of culture medium significantly influences the growth rate and metabolic activity of probiotic bacteria. *L. apis* showed superior biomass accumulation under experimental conditions. These results may be applied to improve functional food production processes and the development of new probiotic formulations.

Keywords: Lactobacillus apis, Bombella apis, biomass production, probiotic microorganisms, culture media optimization

P-19: Exploring Hormetic Effects: Sulforaphane's Role in Triple-Negative Breast Cancer via In Vitro and In Vivo Models

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Our research aimed to investigate how dietary doses of sulforaphane (SFN) impact the proliferation and migration of triple-negative breast cancer (TNBC) cells using both *in vitro* and *in vivo* models. TNBC is a particularly aggressive form of breast cancer with limited treatment options due to the absence of specific drug targets. Natural compounds have garnered significant attention for their potential to enhance cancer treatment effectiveness. Among these compounds, SFN —a natural isothiocyanate—has emerged as a compound with hormetic properties. Depending on its concentration, it can have contrasting effects, either protecting cells or inducing toxicity. In our experiments, we employed MDA-MB-231 cells and utilized a murine TNBC model involving the implantation of 4T1 cells in Balb/c mice. Our *in vivo* investigations revealed that treatment with low dose SFN led to a notable inhibition of tumor growth, with up to a 31% reduction observed. Additionally, we observed decreased cancer cell proliferation, reduced necrotic areas within the tumors, and changes in the types of immune cells present, suggesting a less aggressive tumor phenotype than untreated counterparts. Furthermore, SFN treatment was associated with a decrease in the number of lung metastases. In our *in vitro* studies, we found that a low dose of SFN effectively inhibited the migration of TNBC cells, particularly those derived from 3D spheroids, as opposed to cells cultured in a traditional 2D setting. These findings shed light on the mechanisms by which SFN impacts TNBC cells within their primary tumor environment. The results indicate that the effect of low doses of SFN on TNBC growth is not only related to direct effects on tumor cells, but is also associated with modulation of immune function, modulation of the tumor microenvironment and the process of epithelial-mesenchymal transformation. Overall, our research underscores the potential of SFN as a therapeutic agent for TNBC, highlighting its ability to modulate tumor growth and metastasis.

The research was funded by the National Science Centre, Poland, 2021/41/N/NZ7/02530 and Ministry of Education and Science of the Republic of Poland (statutory activity of National Medicines Institute).

Keywords: triple negative breast cancer, sulforaphane, dietary dose

P-20: SELENIUM NANOPARTICLES OBTAINED BY THE GREEN AND CHEMICAL SYNTHESIS AS ANTIBACTERIAL AGENTS

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The increasing resistance of bacteria to antibiotics has become one of the major problems of modern medicine. Effective alternatives to antibiotics are being intensively sought. Nanoparticles of Ag, Au, Pt, Cu, Ti, or Se have recently become a promising alternative due to their antimicrobial properties. Nanoparticles are usually obtained by chemical synthesis, but this process is costly and not always safe because of the waste generated, which is difficult to dispose of. As a result, eco-friendly synthesis methods are being developed that reduce the generation of harmful by-products. Studies conducted have demonstrated the antibacterial activity of selenium nanoparticles (SeNPs) synthesised by classical chemical methods and green synthesis using plant extracts derived from native plants that show therapeutic potential. These SeNPs have been characterised for their unique chemical properties. Antibacterial and antibiofilm activities of the obtained SeNPs, against Gram-positive and Gram-negative bacteria have been demonstrated. One of the mechanisms of action of selenium nanoparticles is the formation of large amounts of ROS in cells. The response of *E. coli* cells to osmotic and oxidative shock in the presence of selenium nanoparticles obtained by green synthesis and chemical methods was investigated. The inhibition of key mechanisms that allow cell survival under stress conditions was demonstrated, leading to cell death. The results proved that both nanoparticles obtained by chemical synthesis and green synthesis exhibit antimicrobial activity. By disrupting the defence processes of bacteria, they negatively affected their ability to counteract stress, consequently leading to their death.

This research was funded by University of Warsaw Nowe Idee 3b POB I grant numer 501-D355-20-1004310.

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[2] Grudniak A. et al. (2025) Mechanism of Antioxidant Activity of Selenium Nanoparticles Obtained by Green and Chemical Synthesis. International Journal of Nanomedicine 20: 2797-2811.

Keywords: Antibiotic resistance, Selenium nanoparticles, Antimicrobial properties, Green synthesis, Osmotic shock, Oxidative stress

P-21: Effects of transient oxygen and glucose deficiency on rat oligodendroglial mitochondria in in vitro model of neonatal hypoxia-ischemia

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Perinatal asphyxia (PA) is associated with an inadequate oxygenation of tissues before, during or shortly after birth, leading to hypoxic-ischemic brain injury (HI). According to the WHO, the PA consequences contribute to nearly 40% of deaths in children under 5 years of age and are major risk factors for neurodevelopmental disorders in children who have experienced HI. As the HI significantly affects cellular bioenergetics, mitochondria appear to be a potential target for neuroprotective therapies. The detailed mechanisms of mitochondrial reactivity to HI within hours/days after insults remain however to be investigated in neonatal glial cells. To address this issue, the changes in mitochondrial DNA (mtDNA) content in neonatal rat oligodendrocytes (OLs) were determined. Primary mixed glial cultures were obtained from neonatal Wistar rats and used to isolate OL precursors after 12 days of primary culture. Cells cultured in physiological normoxia were subjected to transient oxygen and glucose deprivation (OGD) to mimic HI event in vitro. At the selected time points (i.e. 3, 24, 72, 120 h after OGD), OLs were harvested for molecular analysis and electron microscopy imaging. The PCR results reveal an initial increase in the mtDNA/nDNA (mitochondrial DNA to nuclear DNA) ratio in the first hours after OGD with a peak between the 24 and 48h, followed by a gradual decrease in this ratio at subsequent time points. At 48h and 120h points, respectively, the ratio of mtDNA/nDNA in the OGD group was higher compared to the control group. This may indicate a two-stage reorganization of mitochondria in OLs in response to an OGD event. Cell imaging by means of electron microscopy 24 h after OGD reveals the increased mitophagy process and reorganization of mitochondria. Description of processes initiated in mitochondria after OGD in oligodendrocytes might contribute to identifying the optimal therapeutic window for neuroprotective strategies targeting mitochondria in neonatal OLs.

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Keywords: perinatal asphyxia, hypoxia-ischemia, oxygen and glucose deprivation, oligodendrocytes

P-22: Toxic potential of Bacillus cereus sensu lato strains isolated from food

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Bacillus cereus sensu lato are Gram-positive, facultatively anaerobic bacteria widely distributed in the natural environment, from where they can easily enter food products. Some species within this group may cause foodborne illnesses in humans due to their ability to produce emetic toxin and various enterotoxins. In this study, I analyzed the potential of selected representatives of *B. cereus sensu lato* to produce emetic toxin, non-hemolytic enterotoxin (NHE), hemolytic enterotoxin (HBL), and cytotoxin K (CytK). Using conventional PCR, gene expression analysis (qPCR), and immunochromatographic assays, I determined that all tested strains harbored the operon responsible for NHE synthesis, while the frequency of genetic determinants for HBL and CytK was 37% and 45%, respectively. Only 2% of the tested isolates possessed the *ces* operon required for emetic toxin synthesis. qPCR analyses revealed substantial relative differences in the expression levels of the toxin genes, showing significant variability compared to the reference strain *B. cereus* ATCC 14579. Notably, culturing under elevated CO_2 conditions (5%) resulted in a several- to over tenfold increase in the expression of diarrheal enterotoxins. The results indicate that the presence of *B. cereus* in food may pose a serious challenge in preventing foodborne illness, underscoring the need for ongoing monitoring. Furthermore, gastrointestinal conditions (such as reduced oxygen availability) correlate with increased enterotoxin gene expression, suggesting an adaptive role for NHE and HBL production.

Keywords: Bacillus cereus, food poisoning, toxicity, qPCR

P-23: Migration and Invasion Properties of Glioblastoma Cells in Hypoxia

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The tumor microenvironment plays a crucial role in modulating glioblastoma (GBM) cell behavior, with oxygen availability being a key factor influencing migration and invasion potential. Hypoxia, a common feature of the GBM, is known to induce adaptive cellular responses that may alter these properties. This study aims to evaluate the effects of hypoxic conditions on the migration and invasion potential of GBM cells. Understanding how oxygen levels affect GBM cell movement may reveal new therapeutic strategies to counteract tumor progression. Using four glioblastoma cell lines (*U251, U87, LN229, GL261*) we have compared the results from Transwell migration and invasion assays, wound healing assays, and confluence monitoring in normoxia (21% O_2) and hypoxia (1% O_2). Cells were seeded onto Transwell inserts and then incubated in hypoxia for 24 hours for the migration assay and 48 hours for the invasion assay, which included Geltrex-coated membranes to mimic matrix penetration. The results were analyzed using ImageJ. Both the rate of wound closure, monitored using time-lapse imaging, as well as cell confluence were monitored by measuring the surface area percentage using JuLI™ Stage (NanoEnTek, Seul, Korea Południowa). The results showed that hypoxia impaired both GBM cell migration and, to a lesser extent, invasion. In Transwell assays, cells exposed to hypoxia exhibited decreased migratory and invasive capacity compared to normoxic conditions, with migration reduced by 16% in *LN229*, 28% in *U87*, and 16% in *U251*, while invasion was slightly changed. Similarly, the scratch assay revealed decreased wound closure rates under hypoxia. Confluence analysis further confirmed that hypoxic conditions led to slower monolayer expansion. These findings highlight that hypoxia primarily suppresses glioblastoma cell migration while having a lesser effect on invasion, emphasizing the importance of oxygen levels in glioblastoma progression and therapy development.

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Keywords: Glioblastoma, Hypoxia, Migration, Invasion

P-24: The effect of cytokines on the antioxidant properties of Polyscias filicifolia (C. Moore ex E. Fourn.) L. H. Bailey (Araliaceae) callus extracts

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Polyscias filicifolia is a member of Araliaceae family. The plant is known for its adaptogenic, anti-inflammatory, antioxidant and antimicrobial activities. Due to these properties, it is used in traditional medicine in Southeast Asia. Extracts of *P. filicifolia* are rich in various biologically active compounds, including phenolic acids and triterpenoid saponins [1]. This study compared the antioxidant properties of callus tissue cultivated on four different media modification. The callus was derived from the leaves of *in vitro*-growing plantlets and cultured on solid SH medium [2] supplemented with 1 mg/L of GA_3, 1 mg/L 2,4-D or 2 mg/L 2,4-D and 0.5 mg/L BAP under light/dark 12/12 h regime for 4 weeks. Next callus tissues were collected, lyophilized and extracted. Resulted extracts were investigated for their antioxidant properties including estimation of the content of total phenolics (TPC), total flavonoids (TFC) and the potential for scavenging of DPPH radical and the determination of antioxidant power by FRAP. Additionally, the phytochemical profile of the extracts was analyzed using LCMS (Liquid Chromatography-Mass Spectrometry) to identify and characterize the various bioactive compounds present. The application of cytokines in the study had the significant effect on TPC, TFC and antioxidant properties of extracts in comparison to the control.

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P-25: Silencing Id1 in glioma-associated microglia using targeted nanocarriers to restore their antitumor function

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Glioblastoma (GBM) is the most often and aggressive primary brain tumor in adults, which due to late diagnosis and lack of efficient therapy remains incurable. GBM is highly infiltrated by microglia (brain resident macrophages), peripheral monocytes and macrophages, which play an important role in tumor proliferation and responses to treatments. These cells accumulate and constitute up to 30% of the tumor mass and influenced by glioma cells undergo a phenotypic shift toward an immunosuppressive, tumor supportive state. A key regulator of this polarization is Id1, a dominant-negative inhibitor of basic helix-loop-helix transcription factors, which is strongly upregulated by glioma secreted molecules. The expression of *ID1* is increased in GBM compared to lower grade gliomas and normal brain. Our analysis confirmed that *Id1* is prominently expressed in macrophages and monocytes. We show that *Id1* expression in microglia is strongly upregulated after co-culture with glioma cells. We utilized nanocarriers to deliver siRNA to glioma activated microglia to silence the expression of *Id1* gene and reverse the protumor phenotype. We used two types of carriers: star-shaped polymers and dendrimers decorated with LinTT1. Star polymers exhibited low efficiency in delivering fluorescently labeled siRNA to microglia, which may be due to formation of aggregates. siRNA-loaded dendrimers efficiently reduced *Id1* expression in microglia induced by co-culture with glioma cells. These nanocarriers cross the blood-brain barrier and are selectively uptaken by myeloid cells. Our results show that dendrimer decorated with LinTT are an efficient method to silence *Id1* expression in microglia and may enable reactivation of their antitumor phenotype. This approach opens new avenues for immunomodulatory nanomedicine in glioma therapy.

Supported by grant iNANOGUN/EuroNanoMed3/2021 from NCBR, Poland

Keywords: glioblastoma, microglia, Id1

P-26: Antibacterial potential of biosurfactants produced by Bacillus subtilis WA51 and their interactions with plant compounds

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The phenomenon of growing resistance to commonly used antibacterial agents has become one of the greatest threats to public health. Therefore, natural substances showing antimicrobial activity are of increasing interest. One of them are biosurfactants produced mainly by aerobic bacteria. These are surface-active compounds reducing surface tension which, like surfactin produced by bacteria of the genus *Bacillus*, inhibit the growth of other bacteria due to the interaction of their amphiphilic molecules with cell membrane phospholipids. Their advantages are biodegradability, low toxicity and stability in extreme conditions, so potentially they can be used in many branches of industry.

The main aim of the conducted research is to check the potential of biosurfactants produced by the psychrotolerant strain *Bacillus subtilis* WA51 to inhibit the growth of selected bacterial pathogens and to investigate their synergistic reactions with plant-derived compounds in the context of antibacterial properties. Firstly, biosurfactants were precipitated from the supernatant collected from the *B. subtilis* WA51 culture. The next step was to test the antimicrobial activity using a diffusion method with metal cylinders to obtain inhibition zone. Interactions between biosurfactants and plant-derived compounds (usnic acid, carnosic acid and xanthohumol) were studied using the checkerboard method. Based on the determined fractional inhibitory concentration (FIC), their activity was classified.

The conducted studies revealed that biosurfactants from *B. subtilis* WA51 cultures inhibit the growth of Gram-positive bacteria, particularly those belonging to the genus *Listeria*. However, no inhibitory effect was observed against the tested Gram-negative bacteria. In one case an interaction between the biosurfactants and the applied plant compound was found. In the remaining cases, no such effect was observed. These findings suggest that the biosurfactants produced by *B. subtilis* WA51 exhibit notable antimicrobial activity.

Keywords: biosurfactant, Bacillus subtilis, antibacterial activity

P-27: Identifying molecular determinants of bacterial adhesion to biotic and abiotic surfaces in Ochrobactrum spp.

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Adhesion is the initial step of surface colonization by bacteria. Attachment to surfaces and biofilm formation enhance bacterial survival by protecting them from mechanical damage, facilitating nutrient uptake, and enhancing xenobiotic resistance. To facilitate adhesion, bacteria develop appendages such as pili and flagella, express protein adhesins, and secrete exopolymeric substances. The attachment efficiency further depends on the properties of the surface, such as topography, stiffness, and chemical composition. In this study, we aim to determine the mechanisms involved in attachment to different types of surfaces in *Ochrobactrum* spp. bacteria. Our model system involves two strains, *O. anthropi,* and *O. quorumnocens*. Both strains can colonize plant roots (a biotic surface), but only *O. anthropi* can colonize synthetic abiotic surfaces. To investigate the underlying mechanisms, we generated a pool of mutants using two complementary approaches: random transposon mutagenesis, which allows unbiased identification of novel genetic determinants, and targeted mutagenesis to test candidate genes selected based on literature. The obtained mutants undergo screening for altered adhesion compared to the wild-type strains. The screening assays involve biofilm formation on plastic plates and in medical tubing, and experiments on plant seedlings. Although the study is still ongoing, we have already selected promising mutants with altered adhesion-related phenotypes. An important part of the study involved optimizing molecular tools for genetic manipulation of the tested *Ochrobactrum* spp. strains - a challenging task in non-model microorganisms with broad-spectrum resistance to antibiotics. To conclude, this study presents our model, methodologies, and initial findings in investigating the mechanisms of surface attachment in *Ochrobactrum* spp. We anticipate that these results will contribute to a deeper understanding of the adhesion mechanisms in Alphaproteobacteria.

Keywords: Adhesion, Biofilm, Mutants

P-28: Visualisation of hypoxia-induced changes in cellular NAD+ levels

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Hypoxia alters metabolic pathways and disrupts cellular energy balance. Changes in the levels of NAD+, a central cofactor in redox reactions and metabolic signalling, during hypoxia can affect key processes such as glycolysis, mitochondrial function, cellular response to DNA damage and survivability. The impact of limited oxygen availability highlights the importance of studying NAD+ under these conditions.

In this study, we used HEK 293T cells with stable expression of a nuclear-localized FRET-based biosensor (Nuc-NS-Grapefruit) to investigate NAD+ fluctuations during hypoxia and subsequent reoxygenation. The sensor utilizes mNeonGreen, which emits green fluorescence independently of NAD+, and mScarlet-I, which is excited, via energy transfer (FRET), when NAD+ binds to the sensor and emits red fluorescence. Due to cell-to-cell variation of the probe concentration, we used ratiometric measurement of the mScarlet-I to mNeonGreen emission intensity. Hypoxia was induced by restricting oxygen diffusion during microscopic observation and real-time changes in NAD+ levels were monitored using confocal microscopy.

Our research provides new insights into the real-time dynamics of cellular NAD+ during induced hypoxic stress and subsequent recovery. During hypoxia, we observed a significant decrease in mScarlet-I (red) fluorescence, reflecting decreased NAD+ availability. After reoxygenation, mScarlet-I fluorescence slowly recovered, indicating a return to the original NAD+ levels.

Acknowledgements: Plasmids utilized in this work were generously provided by dr. Nowak and prof. Józkowicz.

Keywords: hypoxia, NAD+, FRET-based biosensor, real-time monitoring

P-29: In vitro evaluation of wild strawberry leaf and acerola fruit extracts: effects of NaOH and AgNPs enrichment on macrophage-mediated inflammation

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Introduction Wild strawberry (*Fragaria vesca L.*) and acerola (*Malpighia emarginata L.*) are rich in anti-inflammatory polyphenols. Enhancing their bioactivity with sodium hydroxide (NaOH) or silver nanoparticles (AgNPs) may improve therapeutic effects.

Objective This study aimed to evaluate the anti-inflammatory effects of wild strawberry leaf extract (WSLE) and acerola fruit extract (AFE), alone or in combination with NaOH or AgNPs enrichment by assessing nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Additionally, extract cytotoxicity was assessed.

Methodology Macrophages were stimulated with LPS and treated with various extracts at 10-200 μg/ml. NO production was measured by the Griess assay, and cell viability by the NRU assay.

Results LPS significantly increased NO production in macrophages, confirming inflammation. WSLE and AFE reduced NO levels at 10-200 μg/ml, though not significantly. Both extracts improved cell viability, with significant increases at 10 and 50 μg/ml WSLE ($p<0.01$, $p<0.05$) and AFE ($p<0.01$, $p<0.05$), as well as at 200 μg/ml AFE ($p<0.05$). NaOH-enriched extracts significantly reduced NO at all concentrations ($p<0.05$) without affecting viability. AgNP-enriched extracts also lowered NO significantly, notably at 10 and 50 μg/ml WSLE+AgNPs ($p<0.01$, $p<0.05$) and 50 and 100 μg/ml AFE+AgNPs ($p<0.05$). WSLE+AgNPs at 200 μg/ml increased viability, while lower concentrations of AFE+AgNPs (10-100 μg/ml) slightly reduced it. Significant differences were found between treatment groups ($p<0.05$).

Conclusions WSLE and AFE reduced NO production in LPS-stimulated macrophages, while NaOH and AgNP enrichment enhanced these effects. The extracts were shown to be non-toxic, as they did not reduce cell viability. These plant extracts showed anti-inflammatory activity, supporting further research into their potential use in conditions such as IBD.

This study is supported by a grant from the Medical University of Lodz (#503/1-156-04/503-11-001 to J Fichna)

Keywords: Anti-inflammatory polyphenols, Inflammatory bowel disease (IBD), Macrophages, Immune modulation

P-30: Microplatform for vascularization of three-dimensional cardiac cell cultures

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The study aimed to design and evaluate a microplatform, which could be used to conduct research about vascularization of three-dimensional cell cultures. The increasing prevalence of cardiovascular diseases highlights the importance of developing advanced *in vitro* models to study human heart. In the first step of the research the microplatform was designed and created using 3D printing technology and polydimethylsiloxane (PDMS) casting techniques. The microplatform consisted of 4 wells, which were used to culture aggregates. Cardiac cell aggregates were prepared using varying ratios of human cardiac fibroblasts (HCF), induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) and human umbilical vein endothelial cells (HUVEC). The aggregates were cultured on agarose platforms, formation of spherical aggregates was observed on the first day. On the third day, the aggregates were transferred to the microplatform wells and embedded in collagen hydrogel. Microscopic imaging revealed morphological changes in the aggregates over time. Fibroblasts exhibited extensive proliferation and migration, leading to the formation of irregular aggregate shapes and blurred boundaries. Notably, the aggregates exhibited spontaneous contractions, indicative of functional cardiomyocyte behavior. Fluorescence imaging demonstrated formation of localized cell clusters and connections between HUVEC cells, suggesting potential early-stage vascularization. To further evaluate vascularization, VEGF (vascular endothelial growth factor) levels were measured in the culture media using ELISA assay. The results showed that HUVEC cells in the aggregates consumed significant amounts of VEGF, indicating active angiogenic processes. The addition of exogenous VEGF to the culture media is recommended to enhance vascularization in future studies.

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Keywords: cardiovascular diseases, stem cells, cardiomyocyte maturation

P-31: Bacterial cellulose as an alternative protectant in the microorganism storage process.

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Yeast, bacteria, and sourdough play an important role in everyday life; however, their effective drying and storage remain a challenge. Various techniques are used to enhance the resistance of cells to different stress factors, such as high temperature, dehydration, oxidation, and osmotic pressure changes. One commonly used approach is the addition of protective substances before the drying process. Bacterial cellulose (BC) is composed of thousands of glucan chains forming a nanoporous structure, which enables it to trap microbial cells. Due to its unique properties, BC is widely used in the industry. The aim of this study was to evaluate the potential use of BC as a protective substance in the drying process of microorganisms. Three microorganisms—*Lactobacillus brevis, Yarrowia lipolytica*, and *Saccharomyces cerevisiae*—were cultured in liquid media. Microorganism immobilization was carried out using two methods: purified BC discs were placed in the cell culture (a) at the time of inoculation and (b) after 24 hours of culture, with both cultures left for an additional 24 hours. Some of the immobilized BC discs were dried at 50°C and 60°C and then digested with cellulase, while others were first digested with cellulase and then dried. It was observed that more cells immobilized on BC when it was added at the time of inoculation. The viability of *L. brevis* cells remained high, whereas the other microorganisms did not exhibit the same level of resistance to drying with BC.

Keywords: microorganism, bacterial cellulose, storage

P-32: Studies on the mitochondrial genome of holoparasitic plants from Orobanchaceae family

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Orobanchaceae is a family of angiosperms that includes species ranging from fully autotrophic to completely heterotrophic and dependent on their hosts (holoparasites). Therefore, they seem to be a promising material for studies on parasitism and the influence of such lifestyle on different aspects of genome evolution, including horizontal gene transfer (HGT). HGT is a phenomenon of the nonsexual transfer of genetic material even between distantly related species, across strong species boundaries. Intimate cellular contacts between organisms, as in host-parasite relationships, may facilitate gene transfer. These reasons inspired us to study mitochondrial genomes of plant holoparasites from the Orobanchaceae family - *Phelipanche ramosa* and *Orobanche coerulescens*. We present preliminary results of the studies: the complex architecture of the mitogenome, mitochondrial genes content and conclusions regarding their activity and potential pseudogenization. The essential step was to obtain the mtDNA sequences with use of two technologies: Oxford Nanopore which delivered longer reads, and Illumina which provided lower error rate. Combined results, with the use of bioinformatics software, allowed us to assemble and analyze both mitogenomes. Additionally, Illumina RNA-Seq transcriptomes sequencing allowed us to check the activity of mitochondrial genes. The results indicate that mitochondrial genomes of both studied species are relatively large. The 1153 kb *O. coerulescens* mitogenome consists of four circular DNA molecules, while the 953 kb *P. ramosa* mitogenome consists of six scaffolds with a total length of 731 bp and two smaller, circular molecules. Gene content analysis suggests a complete lack of *rps2*, *rps7*, *rps11* and *rps19* in the mitogenomes of both species and a lack of *rpl2* and *rps1* in the mitogenome of *O. coerulescens*, which was confirmed by transcriptome analysis. Additionally, *rpl2* and *sdh4* genes are probably partially degraded in *P. ramosa*. Possible traces of HGT are currently being investigated in detail.

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Keywords: HGT, parasitism, mitochondria, mtDNA, evolution

P-33: Development of a highly stable and soluble variant of FGF8 for biomedical applications

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Fibroblast growth factor 8 (FGF8) is a highly labile protein that accumulates into insoluble inclusion bodies when expressed in the bacterial system. The goal of this research was to develop a variant of FGF8 with improved thermodynamic stability, as well as solubility, that could be used for therapeutic purposes. We designed a series of mutations and obtained stable variants, the best of which (M9X), has a denaturation temperature more than 37°C higher than the wild type. Moreover, the M9X protein is highly soluble, allowing it to be efficiently produced in recombinant form in the Escherichia coli expression system and easily purified from the soluble fraction. The hyper stable variant of FGF8 is fully biologically active, exhibiting proliferative, migratory and anti-apoptotic properties. In addition, the introduction of substitutions that lowered FGF8's affinity for heparan sulfate increases the protein's availability to target cells, enhancing its potential for medical applications.

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Keywords: fibroblast growth factors, FGF, FGF8, thermodynamic stability, solubility, signalling

P-34: Exploring the pleiotropic effects of isoxazole derivative of usnic acid in breast cancer cells

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Breast cancer is one of the most common cancers among women worldwide. Increasing evidence suggests that Wnt signaling pathway plays a key role in breast cancer chemo- and multidrug resistance, stem cell features, and metastasis. It has been shown that usnic acid derivative 2b potently decreased the proliferative potential of MCF-7 breast cancer which correlated with endoplasmic reticulum stress and paraptosis-like cell death. As RNA-seq analysis indicated a drop in some Wnt pathway genes in 2b-treated MCF-7 cells, the present study aimed to investigate the impact of 2b on processes regulated by this pathway in breast cancer cells representing different molecular subtypes (luminal, HER2-enriched and triple-negative). Analysis of transcriptome (RNA-seq and qRT-PCR) revealed that 2b induced dose- and time-dependent alterations in the transcript profile of WNT genes. Among them, *LRP5* and *LRP6* levels are markedly down-regulated in MDA-MB-231 and MCF-7 cells. Moreover, confocal microscopy analysis revealed that 2b significantly decreased β-catenin levels in the nucleus compared to control cells. Using Matrigel 3D cultures, we observed that spheroids lost their spherical shape, decreased in size, and began to disintegrate. This effect was particularly visible in the SKBR-3 and MDA-MB-231 cell lines. Additionally, 2b inhibited the migration of breast cancer cells. Cell migration (wound healing assay) was imaged on the Holomonitor (HM) for 72 hours. Complete wound closure was observed in the control group, compared to 32% and 17% coverage areas in 2b-treated MCF-7 and MDA-MB-231 cells, respectively. In addition, 2b reduced the clonogenic potential of these two tumor cell lines. Together, these results indicate that 2b is a promising agent for breast cancer treatment.

This work was supported by the National Science Centre, Poland (2017/26/M/NZ7/00668).

Keywords: breast cancer, usnic acid, WNT signaling pathway

P-35: Same Microbial Oil, Different Outcome? The Impact of Extraction Methods on Composition of oleaginous yeast lipids

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Yarrowia lipolytica is an oleaginous microorganism capable of synthesizing oil, which can constitute more than 20% of its dry biomass. This well-studied microorganism has been widely researched by scientists. Extracted microbial oil has the potential to serve as an innovative base for dietary supplements. Yarrowia lipolytica utilizes two types of carbon sources: hydrophilic (sugars) and hydrophobic (oils). This metabolic flexibility can be applied to the valorization and utilization of waste oils from the food industry. In this study, there was conducted a series of experiments and analyses to investigate the effect of different extraction methods on microbial oil composition. Y. lipolytica was cultivated in a mineral medium with nitrogen source limitation using waste oil from the university's student canteen as the sole carbon source. The fermentation was carried out at 26°C for four days. After cultivation, the total biomass was dried, and the oil was extracted using three different methods: supercritical CO_2 extraction, cryogenic milling, and Soxhlet extraction. The extracted oil was analyzed for free fatty acid (FFA) composition, oxidative stability with pressure differential scanning calorimetry (PDSC), antioxidant activity, and polycyclic aromatic hydrocarbons content (PAHs). The results were compared to determine whether the extraction method influences the composition of the obtained microbial oil.

The project financed by Polish Government Ministry of Education program „Studenckie koła naukowe tworzą innowacje" (SKN/SP/601167/2024).

Keywords: Yarrowia lipolytica, carbon dioxide supercritical extraction, single cell oils

P-36: The influence of chemotherapeutic stress and hypoxia on the metabolic plasticity of human glioblastoma multiforme

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Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor. The high heterogeneity, plasticity, and adaptive capacities of GBM account for its high treatment resistance. Conventional therapies are ineffective and GBM patients develop resistance to commonly used chemotherapeutics. One of the promising candidates for GBM therapy is doxorubicin (DOX), which is an anthracycline antibiotic with cytostatic activity. It inhibits the cell cycle and induces metabolic dysfunctions, leading to cell death. However, its action in hypoxic conditions, which is characteristic of neoplastic tumors *in vivo*, remains poorly described. This study aimed to determine the effect of hypoxia on the metabolic reprogramming of DOX-treated T98G and U87-MG cells.

For this purpose, T98G and U87-MG cells were pulse-treated (for 24 hours) with 1 μM/100 nM DOX (T98G/U87 cells, respectively) or continuously exposed to 5 nM DOX in standard conditions (normoxia) or hypoxia (1% O_2). Levels and cellular localization of selected metabolic markers were assessed using confocal microscopy. The activity of mitochondrial enzymes was evaluated using the MTT assay, concomitantly with the estimation of ATP and lactate levels with dedicated biochemical assays.

DOX and hypoxia significantly altered the levels and intracellular localization of glucose transporter 1 (GLUT1), hexokinase 1 (HK1), glutaminase (GLS), carnitine palmitoyltransferase II (CPT2), isocitrate dehydrogenase 1 (IDH1) and ATP synthase. They also affected the levels of energy carriers. In parallel, the changes in the levels and localization of hypoxia-inducible factor 1 (HIF-1α) and Ki67 (considered a marker of cell proliferation) were observed. These effects were more pronounced in the presence of higher DOX concentrations.

Our findings highlight the impact of combined chemotherapeutic and metabolic stress on GBM metabolic plasticity, providing a basis for further research into mechanisms of adaptation and chemoresistance.

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Keywords: glioblastoma multiforme, metabolic plasticity, doxorubicin, hypoxia

P-37: Investigation of the transient increase in blood-brain barrier permeability using a microsystem.

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The blood-brain barrier (BBB) is a highly selective interface between the circulatory system and the central nervous system (CNS), playing a fundamental role in maintaining CNS homeostasis. However, it also represents a substantial obstacle to the effective delivery of therapeutic agents to the brain [1]. Consequently, the development of minimally invasive strategies to transiently enhance BBB permeability is of critical importance for advancing the treatment of neurological disorders. This study employs a physiologically relevant three-dimensional *in vitro* BBB model that incorporates five key cell types—endothelial cells, pericytes, astrocytes, microglia, and neurons—thereby closely replicating the cellular composition of the BBB. This model enables the precise administration of electrical pulses and facilitates the systematic evaluation of barrier integrity under various electroporation conditions. By modulating pulse parameters, this study seeks to establish the optimal conditions for transient BBB permeability enhancement while preserving cellular viability and ensuring the restoration of barrier function. Electroporation is a minimally invasive technique with well-established clinical applications, including its use in oncology and gene therapy. It offers a highly controlled and reproducible method for temporarily increasing BBB permeability, thereby presenting significant potential for improving drug delivery to the CNS while maintaining overall barrier integrity [2]. By systematically optimizing electroporation parameters, this research aims to elucidate their impact on BBB integrity and to define conditions that achieve an optimal balance between enhanced permeability, cellular viability, and functional recovery of the barrier. The findings of this study, including details of the experimental set-up, methodological approach, and preliminary results, will be presented in the poster.

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Keywords: blood-brain barrier, electroporation, drug delivery, in vitro model

P-38: Biosensing albumin with new benzylidene derivatives - investigation of fluorophore-protein interactions

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Albumins are the most common proteins in blood plasma. They play an important role in the transportation of various substances in the body, such as amino acids, fatty acids, drugs, or metabolites, thanks to active sites capable of binding ligands. Albumin levels in the blood are an indicator of health, and their imbalances may indicate various diseases, such as liver disease, kidney disease, malnutrition, or diabetes. Bovine serum albumin (BSA) is a well-studied model protein with a structure homologous to human serum albumin, and it is widely used in research, including protein and ligand binding. In its structure, it has two tryptophan residues responsible for the internal fluorescence of the protein, which can be quenched by various fluorophores. Spectroscopic detection of proteins is simple, fast, and selective compared to other methods used. Hence, they are widely studied and have found application in fields such as medicine, diagnostics, and biochemistry. Spectroscopic studies of benzylidene derivatives and studies of the interactions of these derivatives with bovine serum albumin have been carried out. Fluorophore-BSA interactions allowed us to determine binding parameters such as the number of binding sites, binding constant, Stern-Volmer constant, and molecular quenching constant.

Research financed by the project funded by the Ministry of Science and Higher Education entitled: 'Students scientific associations create innovations' contract number SKN/SP/602770/2024.

Keywords: benzylidene derivatives, bovine serum albumin (BSA), molecular interactions

P-39: Elevated glucose levels reduce the degradation of amyloid precursor protein (APP)

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In recent years, numerous studies have explored the relationship between diabetes mellitus and neurodegenerative diseases, such as Alzheimer's disease (AD). One hallmark of AD is an elevated level of amyloid precursor protein (APP) in cells. APP can be converted into β-amyloid, which can accumulate in the extracellular space of neurons, ultimately leading to their death. Therefore, proper control of the quantity and quality of APP is crucial for organisms. APP is primarily degraded in lysosomes through autophagy; however, misfolded and potentially amyloidogenic APP is degraded by the 26S proteasome. The step preceding proteasomal degradation involves the recognition of APP as abnormal in the endoplasmic reticulum (ER) and its transport from the ER to the cytosol via the ER-associated degradation (ERAD) pathway. Previous studies have confirmed that a high glucose concentration impairs APP degradation; however, the exact process has not yet been identified. The aim of the project is to investigate the effect of glucose levels on intracellular APP metabolism. We examined the effect of various glucose concentrations on the efficiency of lysosomal and proteasomal APP degradation. Human embryonic kidney (HEK293) cells were cultured for 72 hours at glucose concentrations of 5. 5 mM, 10 mM, and 25 mM, respectively. Moreover, cells were treated with inhibitors of two major APP degradation pathways: epoxomicin or bafilomycin. To confirm that glucose concentration affects the degradation of APP rather than its expression, real-time PCR analysis was performed. The results prove that a high glucose level interferes with the proteasomal degradation of endogenous APP. On the other hand, its impact on lysosomal APP degradation cannot be ruled out. The presented results are not the final conclusions of the project, and the research will be continued.

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Keywords: amyloid precursor protein, Alzheimer's disease, diabetes mellitus, proteasomal degradation, lysosomal degradation

P-40: Microbial hitchhikers: How pollinators shape nectar microbiomes across plant species

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Pollination is one of the most important ecological interactions, without it many plant populationswould decline and eventually disappear. To protect biodiversity, human well-being and the economywe need a robust assessment of factors influencing pollination, and one of them are nectar microbialcommunities. The presence of microorganisms in nectar is a common and important phenomenon. Moreover, every living organism, including pollinators, has its microbiota. Current research onmicroorganisms in nectar shows their great role in pollination efficiency, plant and pollinator fitness. Surprisingly, many of the fundamental views concerning microorganisms in nectar are untestedhypotheses. Our project addresses crucial gaps concerning the role of floral traits and pollinators inshaping nectar microbiome. We collected nectar from 10 species of flowering plants with differentflower features, where we examined nectar properties and nectar microbiome. We also recordedplant-pollinator interaction. We hypothesize that plant species visited by the same types ofpollinators may support pollinator-linked specific nectar microorganisms communities. Our resultsindicate the important role of pollinators with a smaller influence of flower traits and nectarproperties.

Keywords: plants, pollinators, microbiome, floral nectar, floral traits

P-41: Studying the role of LINC00116 in lymphomas: Does it function as a long non-coding RNA?

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Long non-coding RNAs (lncRNAs) are defined as more than 200 nucleotides long RNAs that do not have the potential to encode proteins, but can regulate gene expression and protein functions. However, developments in bioinformatics have allowed the discovery of small open reading frames (smORFs) in the lncRNA sequences that enable translation into micropeptides. One of such bifunctional lncRNAs is LINC00116, which is translated to a 56-amino-acid Mitoregulin (MTLN) peptide located in the mitochondrial membrane. LINC00116 shows increased levels in B-cell lymphomas compared to B-lymphocytes suggesting its oncogenic potential. However, the function of LINC00116 and MTLN in B-cell lymphoma is unknown. The aim of this project is to investigate whether LINC00116 and MTLN have separate cellular functions in B-cell lymphoma cells. We created genetic constructs to selectively overexpress LINC00116 or MTLN peptide in L428 and SUDHL5 lymphoma cells using lentiviral vectors. We confirmed the overexpression of LINC00116 by qRT-PCR and overexpression of MTLN peptide by Western Blot. The lentiviral vectors contained the gene encoding the green fluorescence protein (GFP), which allowed cytometric analysis of transduced cells within 21 days. The percentage of L428 cells with overexpressed MTLN was decreased compared to cocultured wild-type cells. This decrease was not observed when we overexpressed LINC00116 without MTLN-coding potential. In conclusion, we created genetic constructs that selectively overexpressed LINC00116 or MTLN in lymphoma cells and we observed decreased growth of lymphoma cells with overexpressed MTLN. Further studies to demonstrate possible differences in LINC00116 and MTLN functions in B-cell lymphoma are ongoing.

12th funding competition for Project-Based Learning (PBL) under the Excellence Initiative - Research University program, Silesian University of Technology.

Keywords: Long non-coding RNAs, lymphoma, micropeptides

P-42: TRIM21 as a molecular link between inflammation and metabolic dysregulation in diabetes

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TRIM21 (tripartite motif-containing protein 21) is an E3 ubiquitin ligase that plays a crucial role in the immune response and other important processes by activating intracellular signaling pathways and regulating protein stability. Increasing evidence suggests its significant but complex role in the pathogenesis of diabetes and related organ complications. Under chronic hyperglycemic conditions, the upregulation of TRIM21 promotes the proteasomal degradation of the transcription factor FOXD1, leading to reduced BCL-2 expression and enhanced apoptosis in human endothelial and hepatic cells.

Contrarily, in type 2 diabetes, reduced TRIM21 expression in the liver has been observed and is probably associated with impaired glucose and lipid metabolism. These effects are mediated by the ubiquitination and degradation of key regulatory proteins, influencing metabolic balance. The mechanism of decreased TRIM21 expression in the liver remains unknown; however, factors regulating the metabolic function of the liver and metabolites to which hepatic cells are exposed in obese, insulin-resistant, or diabetic patients may be involved in this process.

To explore the mechanisms regulating TRIM21 expression in hepatic cells, we conducted an *in vitro* study using the human hepatocellular carcinoma cell line HepG2. Cells were cultured under varying glucose levels and stimulated with interferon (IFN-γ), a known inducer of TRIM21 expression in various cells, or with insulin (INS) for 24 and 48 hours. Subsequently, TRIM21 transcript levels were measured using qPCR.

Our studies aim to elucidate the biochemical mechanism underlying the regulation of TRIM21 in hepatocytes.

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Keywords: TRIM21, diabetes, apoptosis, qPCR

P-43: The Importance of AlkB Deoxygenate and AidB Dehydrogenase in the Repair of Damaged RNA Bases

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AidB dehydrogenase and AlkB dioxygenase are induced within *E. coli* adaptive response to low doses of methylating agents. AlkB belongs to the superfamily of 2-oxoglutarate- and iron-dependent dioxygenases, which remove alkyl lesions from bases *via* an oxidative mechanism restoring native DNA or RNA structure, but toxic and mutagenic byproducts, such as formaldehyde, glyoxal, and malondialdehyde, are generated. We have recently confirmed that AlkB repairs *in vitro* etheno- and hydroxypropanoadducts to RNA bases. The function and substrates of AidB have yet to be discovered.

Our research aims to investigate whether AlkB could repair in vivo RNA lessons other than methylated ones. We analyzed the substrate specificity of AlkB dioxygenase towards etheno- and hydroxypropano-adducts formed in RNA under the influence of chloroacetaldehyde and acrolein, respectively. Additionally, we studied the potential role of AidB dehydrogenase in maintaining the byproducts of the AlkB dioxygenase repair reaction.

Using the disk diffusion method, we evaluated the sensitivity to mutagenic agents in *wt*, *alkB*, *aidB*, and *alkBaidB* strains. We found that strains lacking repair activities were not significantly more sensitive to exogenously administered formaldehyde, glyoxal, or malondialdehyde than wild-type strains.

As an *in vivo* RNA damage repair model, we used bacteriophage MS2, whose genetic material is single-stranded RNA. We modified phage particles with various concentrations of mutagens. MS2 survival decreased with increasing concentrations of these compounds. No significant differences in survival were observed between the wild-type strain and its derivatives lacking AlkB and AidB activities. However, defective strains were more sensitive to the methylating agent than wild-type strains.

We conclude that we observed the cytotoxic effect of studied compounds in both experiments, which was more potent than mutagenic.

Similar to repairing DNA damage, AlkB may be responsible for repairing endogenously occurring RNA damage.

The role and cooperation of AidB dehydrogenase and AlkB dioxygenase in repair of adducts to DNA and RNA bases. 2018/29/B/NZ3/02285

Keywords: AlkB, AidB, RNA repair, mutagenic compounds, adaptive response

P-44: Innovative Iridium(III) Complexes as Theranostic Photosensitizers for Photodynamic Therapy (PDT) and Sensors for High-Precision Single-Cell Imaging.

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In recent years, there has been a significant rise in cancer incidence and mortality,highlighting the urgent need for advancements in both cancer diagnostics and treatmentstrategies. Iridium(III) complexes have emerged as promising candidates for thedevelopment of next-generation photosensitizers for photodynamic therapy (PDT) and ashighly precise imaging sensors at the single-cell level. Due to their distinctive photophysicaland photochemical properties, these complexes hold great potential in theranostics, a fieldthat integrates therapeutic and diagnostic applications. Photodynamic therapy (PDT) is widely used in the treatment of cancer and certain dermatological conditions. The process relies on three key components: a photosensitizer, light of a specific wavelength, and molecular oxygen. Upon activation by appropriate light exposure, iridium(III) complexes generate reactive oxygen species (ROS), which induce cellular damage and ultimately lead to cancer cell death. As a novel and versatile class of compounds, iridium(III) complexes offer significant potential in both PDT and advanced cellular imaging. Therefore, this study explored newly synthesized iridium(III) complexes to evaluate their properties and applications. The primary aim of this research was to assess their cytotoxicity. The study was conducted using an in vitro model with cancer cell lines. Cytotoxicity was determined through the MTT assay, a colorimetric method that evaluates the enzymatic activity of mitochondrial-aldehydrogenases. Additionally, spectrophotometric and spectroscopic analyses were performed to further characterize the photophysical properties of the iridium(III) complexes.

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Keywords: iridium (III) complexes, cytotoxicity, cancer cell, photodynamic terapy

P-45: *Sarracenia purpurea* L.: from carnivorous to medicinal

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The medicinal properties of insectivorous plants have been known for centuries and historical data indicate that infusions of these plants have been used to treat various ailments. This information inspired the authors to undertake research on the plant *Sarracenia purpurea* L. The plant was introduced into *in vitro* cultures, growth conditions were optimized and transformation was carried out using the bacteria *Rhizobium rhizogenes*, which has the ability to induce hairy root formation in plants. The antioxidant activity and total content of selected secondary metabolites with anticancer potential were determined in the obtained composite plants. The results showed that the obtained composite plant *Sarracenia purpurea* L. has significant antioxidant activity and an interesting content of secondary metabolites. This may suggest that the plant has pharmacological potential. These results provide a promising basis for further research to further analyze and isolate the compounds responsible for these properties. Research will also be needed to evaluate the anticancer efficacy of these metabolites.

Keywords: Sarracenia purpurea L., in vitro cultures, Rhizobium rhizogenes, composite plant, hairy roots

P-46: ANALYSIS OF SPECIFIC METABOLITES IN SELECTED CARNIVOROUS PLANTS GROWN UNDER IN VIVO CONDITIONS

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Carnivorous plants are a fascinating group of species, known not only for their unique adaptations to nutrient-poor environments, but also for their ability to synthesize a wide array of biologically active compounds. This study analyzes metabolites in chosen species of carnivorous plants grown under *in vivo* conditions. The research is part of a broader project to explore the regenerative capacity and phytochemical profile of carnivorous plant species cultivated *in vitro*, with the *in vivo* study providing baseline data for comparison and validation of *in vitro* findings. Special focus is placed on compounds that may possess therapeutic potential, including anticancer activity. Before metabolite analysis the plant material has been lyophilized to ensure sample stability and preserve the integrity of the compounds. The materials used include representatives of the plant species *Drosera*, *Dionaea*, *Nepenthes*, and *Sarracenia*. The results of this study are expected to contribute to the growing interest in plant-derived metabolites as prospective agents in pharmaceutical and medical applications.

The fifth edition of the Young Minds Project competition at the Wrocław University of Environmental and Life Sciences

Keywords: Carnivorous plants, metabolite profiling, in vivo conditions, therapeutic potential, anticancer compounds, Drosera, Dionaea, Nepenthes, Sarracenia

P-47: Comparative analysis of the efficiency of photothermal therapy using gold nanostars and gold-coated iron oxide nanoparticles

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Photothermal therapy (PTT) is a targeted cancer treatment that utilizes electromagnetic radiation and photoactive agents capable of converting absorbed radiation into heat. When a photoactive agent is introduced into a tumor and subsequently activated, the localized temperature increase induces cell apoptosis. Current research focuses on evaluating the feasibility of using nanoparticles (NPs) as photoactive agents, as they possess unique physicochemical and mechanical properties due to their small size and large specific surface area. This study aimed to compare the efficacy of PTT using two types of NPs: gold nanostars (NSs) and magnetic NPs (1:3 Fe_3O_4:Au) with a nucleolin-modified surface. The biological activity of these NPs was tested on two skin cell lines: normal HaCaT and cancerous A375. Cytotoxicity was assessed using the MTT viability assay after 24 and 48 hours of incubation with NP solutions. Additionally, the energy conversion capacity of the NPs was analyzed by measuring the temperature change in NP solutions following laser irradiation at the wavelength used in the PTT procedure. The effectiveness of PTT was then evaluated based on selected NP concentrations. The results demonstrated that the tested NPs effectively converted energy and, upon photoactivation, significantly reduced cancer cell viability. Furthermore, cancer cell viability decreased to a greater extent than that of normal cells after PTT. These findings confirm the potential applicability of both types of NPs as photoactive agents in PTT.

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Keywords: photothermal theraphy, nanpoparticles, energy conversion efficiency, efficacy of photothermal therapy, cell viability

P-48: The role of Heme Oxygenase 1 in stress granule formation. Reevaluation of Integrated Stress Response

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Disturbance of homeostasis by various types of stress factors induces conserved cellular pathway of integrated stress response (ISR) aimed at adapting to altered conditions and nullifying the deleterious effects of the stressor. At the level of expression, this results in the global arrest of protein biosynthesis at the translation initiation stage. In particular, serine/threonine kinases phosphorylate the alpha subunit of translation initiation factor (eIF2α), thus preventing the ternary translation complex from forming. Here, we aimed to describe the ISR pathway in iPS cells with regard to the activities of heme oxygenase 1 (HO-1 *Hmox1*). We used iPS cells lacking both HO-1 and HO-2 or possessing only HO-1 localised either in the cytoplasm or the nucleus. Using sodium arsenite (SA) stimulation, we observed increased susceptibility for stress granule formation - the hallmark of ISR - in cells lacking HO-1 and with HO-1 overexpressed in the nucleus. The increase of the SA dosage seemed to saturate this effect. We then conducted analogous experiments using proteasome inhibitor - MG-132 and yielded unexpected results of knockout cell lines being free of any stress granule. This is contradictory to the current state of knowledge phrasing shared response pathway between SA and MG-132 treatment with heme-regulated inhibitor kinase (HRI) playing a crucial role in signal transduction. Furthermore, chronic stress stimulation revealed the outstanding viability of cells lacking HO-1 after the inhibition of proteasome. Additionally, the localisation of HO-1 was assessed after stress induction via imaging cytometry and various stainin,g prompting no apparent translocation. This preliminary data suggests a potential shift in the perception of the cytoprotective role of HO-1 during stress response. Ongoing studies are focused on pinpointing the fundamentals of those changes, particularly the role of HRI in the context of localisation and the presence of HO-1.

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Keywords: integrated stress response, IPSC, HO-1, HRI, stress granules

P-49: The role of glutathione in CRISPR-Cas9-modified cells

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Glutathione (GSH) plays a key role in defending cells against oxidative stress, which is an imbalance between the production of reactive oxygen species (ROS) and the ability of cells to neutralize them. The glutathione shield is a key protective mechanism in which several enzymes work together to maintain adequate GSH levels and remove RFTs. It also plays a key role in protecting cells from ferroptosis, a type of programmed cell death that is iron-dependent and characterized by the accumulation of RFTs in cell membrane lipids. Ferroptosis is initiated by the accumulation of lipid peroxides, which are formed because of oxidative stress and are toxic to cells, leading to cell damage and death. The aim of this study was to investigate the role of the glutathione shield in the defense of cells against oxidative stress. The study was conducted on CRISPR/Cas9-modified colon cancer cell lines, as well as on wild-type cells. Cells were treated with erastin at a dose of 10uM to induce ferroptase, then total glutathione levels were checked, and mRNA levels of *GSR*, *GPX4*, *TRX* and *TRXRD* genes were examined by RT-qPCR. A decrease in *TRX* and *TRXRD* expression was observed after erastin treatment in all cell lines. After treatment with erastin, wild-type cells showed a significant increase in transcript levels for *GSR*, which was not observed in the engineered cells. The level of total glutathione in wild-type cells was 2 times lower after treatment with the inducer. In the modified cells with the *GPX4* gene deactivated, the level was observed to be half that of the control relative to the wild-type cells. Deletion of a fragment of the *GPX4* gene caused a decrease in the cellular glutathione pool, due to the absence of the main antioxidant involved in the renewal of the GSH pool. Application of erastin alone caused levels to drop by half in all lines. Understanding the mechanisms of action of the glutathione shield and its role in counteracting ferroptosis is crucial, especially in the context of oxidative stress-related diseases such

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Keywords: Cell, CRISPR/Cas9, Glutathione, GPX4, HCT116, ROS

P-50: Microbial consortium-driven strategies for enhanced sewage sludge composting and environmental impact mitigation

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Backgrounds and Aims: Sewage sludge composting plays a vital role in sustainable waste management and environmental protection. This study investigates the integrated use of a lignocellulolytic fungal consortium, including *Aspergillus sp.*, *Penicillium sp.*, and *Trichoderma sp.*, as a bioformulation and a bacterial consortium including (*Achromobacter* sp., *Bacillus* sp., *Spororacina* sp., *Pseudomonas* 157 sp., and *Ochrobactrum* sp.) capable of degrading proteins, fats, and hydrocarbons to optimize composting efficiency. The aim of this study is to demonstrate how fungal and bacterial bioaugmentation, both alone and together, enhances organic matter mineralization, shapes microbial community dynamics, and improves the overall quality of sewage sludge compost compared to untreated controls.

Methods: The composting experiment was conducted at Hydrogetechnika Sp. z o.o., Kielce, Poland, over an 8-week period. The experiment utilized six variants of composting treatments, including applying fungal and bacterial bioformulations individually and combined. Sewage sludge and wheat straw as a bulking agent, served as the substrate. Various physicochemical parameters and microbiological indicators, along with dehydrogenase activity were monitored throughout the composting process.

Results and conclusions: The results showed that the fungal consortium significantly enhanced lignocellulosic biomass degradation and organic matter mineralization, especially when co-applied with the bacterial preparation. This combination also reduced moisture content and stimulated microbial activity, resulting in superior compost maturity and stability. While bacterial preparations alone showed moderate improvements, the integrated fungal-bacterial strategy accelerated composting efficiency and promoted beneficial microbial succession. This study highlights the advantages of utilizing microbial consortia to enhance sewage sludge composting and improve compost quality under industrial conditions.

Keywords: Sewage sludge composting, Microbial consortia, Bioaugmentation

P-51: Gene expression profile for cysteine cathepsins and resulting TLR-IRF pathways in murine cDC subsets

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Splenic cDC1 and cDC2 cells of BALB/c and C57BL/6 mice show no interstrain differences in gene expression for cysteine cathepsins and resulting TLR-IRF pathways

Dendritic cells (DCs) are the sentinels of the immune system, bridging innate and adaptive immunity. Among them, the conventional DC subsets—cDC1 and cDC2—differ in their antigen-processing strategies and immunomodulatory roles. An essential part of this process involves cathepsins (Cts) - lysosomal proteases that degrade antigens to facilitate antigen presentation. Despite their role in antigen digestion and presentation, cathepsins influence pathogen recognition by modulating Toll-like receptor (TLR) signaling and activating interferon regulatory factors (IRFs) to orchestrate inflammatory responses. Inbred mouse strains exhibit differences in disease progression and severity across a range of infectious diseases. To investigate potential interstrain differences in cathepsin-TLR-IRF-dependent interactions, we analyzed the expression of genes encoding cysteine cathepsins (CtsB, CtsL, CtsS), TLRs (TLR3, TLR7, TLR9), IRFs (IRF3, IRF5, IRF7), and key inflammatory cytokines (IL-1b, IL-18, TNF) in cDC1 and cDC2 cells derived from BALB/c and C57BL/6 mice using qPCR. Surprisingly, despite differences in immune predispositions between these mouse strains, no significant interstrain differences in gene expression were observed. This indicates that cathepsin-TLR-IRF-dependent pathways function similarly in both strains, suggesting a conserved mechanism for antigen processing and immune activation in murine cDC1 and cDC2 cells. These findings contribute to a better understanding of the genetic regulation of DC function across different murine strains.

This work was supported by PRELUDIUM-BIS NCN grant No. UMO-2020/39/O/NZ6/03252.

Keywords: dendritic cells, cDC1, cDC2, cathepsins, TLR, IRF, BALB/c mouse, C57BL/6 mouse

P-52: Purification and characterization of endocrine fibroblast growth factors (FGFs): the example of FGF19

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Endocrine fibroblast growth factors (FGFs), consisting of FGF19, FGF21, and FGF23, play crucial roles in metabolic regulation, including bile acid homeostasis and glucose metabolism. Their biological functions make them promising biotherapeutic agents. However, pharmacokinetic challenges and potential tumorigenicity limit their clinical application. Developing an optimized purification protocol would facilitate the engineering of endocrine FGFs with improved bio-physical properties, addressing existing limitations in their therapeutic use. The objective of this study was to develop a purification protocol for FGF19 with a His-tag expressed in an *E. coli* system. Overexpression conditions were optimized by evaluating the appropriate bacterial strain, culture medium, and growth temperature. Localization analysis confirmed that FGF19 predominantly accumulated in inclusion bodies, rendering purification from the soluble fraction ineffective. Protein refolding attempts under various conditions did not facilitate efficient binding to Ni-NTA resin. Purification was only successful under denaturing conditions (1M guanidine hydrochloride). Buffer exchange from high-imidazole conditions to PBS, followed by incubation with heparin, a natural proteoglycan with low affinity for FGF19, enabled proper protein folding. Correct folding was verified by circular dichroism (CD) analysis, which showed a thermal denaturation curve characteristic of wild-type FGF19. To confirm biological activity, cell culture assays have been planned. The protocol is currently undergoing further optimization and evaluation. As FGF21 and FGF23 exhibit sequence and structural similarities to FGF19 and also localize within inclusion bodies, the feasibility of applying the same purification strategy to these proteins will be explored. A reliable purification protocol for endocrine FGFs will facilitate the production of engineered variants, supporting research into their therapeutic application.

Keywords: protein purification, endocrine FGFs, metabolic disease, biomedical applications

P-53: Biotechnological approach to *Aralia cachemirica* Decne: transformation and in vitro cultivation of hairy roots for biodiversity conservation.

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Introduction *Aralia cachemirica* Decne is a rare Himalayan shrub species, endangered by overharvesting for traditionalmedicine and the effects of climate change. This species has a limited distribution, leading to populationdecline. In vitro cultivation offers a sustainable alternative, providing plant material without depleting wildpopulations. This study explores bacterial transformation of *A. cachemirica* as a method to obtain plantmaterial.

Objective The aim of this study was to establish a sustainable platform for the induction and maintenance of hairy rootcultures of *A. cachemirica*, thereby facilitating in vitro propagation and reducing reliance on naturalpopulations.

Materials and Methods Plantlets of *A. cachemirica* were infected with suspensions of three *Rhizobium rhizogenes* strains: ATCC15834, A4, and LBA 9402. Hairy root lines were selected and cultivated on SH medium. Polymerase chainreaction (PCR) analysis was conducted to confirm successful genetic transformation.

Results This study reports, for the first time, the successful establishment of hairy root cultures in *A. cachemirica*. Root induction was observed following infection with strains ATCC 15834 and A4, whereas strain LBA9402 did not elicit a response. The obtained root lines exhibited considerable morphological variation,ranging from compact, highly branched forms to elongated, unbranched roots.

Conclusion The results confirm the susceptibility of *A. cachemirica* to genetic transformation via *Rhizobium rhizogenes*. The resulting hairy root lines, characterized by rapid growth, represent a promising source of plant biomass. This biotechnological approach holds significant potential for the conservation of biodiversity, particularlywith regard to endemic and endangered plant species.

This work was supported by grant with number 18/F/MG/N/24

Keywords: hairy roots, endemic plant, bacterial transformation, in vitro, biodiversity

P-54: Development of a novel microfluidic chip for thermotaxis-based sperm selection

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According to the WHO, up to 17.5% of adults may be affected by infertility, with male-factor infertility often attributed to unexplained causes or poor semen quality. Such circumstances create an increasing demand for Assisted Reproductive Technologies (ART), which require efficient semen sample preparation and, if necessary, quality enhancement. The selection of fertilizable spermatozoa is crucial for successful in vitro fertilization (IVF). Current sperm selection techniques often require skilled personnel and can compromise sperm quality due to lengthy processing and centrifugation. Microfluidic systems could serve as a promising alternative to current complex and time-consuming methods. Existing microsystems typically focus on maximizing the exploitation of a single phenomenon. This research adopts a different strategy. To increase the effectiveness of sperm selection, an integrated microsystem has been developed that exploits multiple phenomena simultaneously. The system consists of alternating layers of hydrophilic and double-sided adhesive films, with PMMA providing structural support. Membranes with pore sizes optimized to 3 μm are used to filter the semen sample. The dimensions of the microchannels are 0.1 $\times$ 1 $\times$ 65 mm. This easy-to-use device allows for the assessment of sperm self-motility, thermo-, and chemotaxis from a very small sample volume of just 100 μL, without the need for additional equipment. A heated holder is also included, serving as a universal heating plate for microscopy observations of other microfluidic systems and more. As part of this research, various channel geometries and thermotaxis were investigated for their effect on sperm viability and motility. Detailed results will be presented during the poster session. We believe that the newly developed microsystem will facilitate assisted reproductive techniques and contribute to increasing the effectiveness of in vitro fertilization, supporting the fight against infertility.

Keywords: sperm cells, microfluidics, thermotaxis, selection, sperm motility

P-55: Panel design for the study of macrophage activity during the course of sepsis

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Sepsis is the world's leading cause of premature death, taking a toll of many millions each year. Patients diagnosed with sepsis struggle with severe organ damage and extensive inflammation. Macrophages play a crucial role in orchestrating both the pro-inflammatory and anti-inflammatory phases of the septic response, yet their heterogeneity and dynamic changes remain insufficiently understood. In this study, we aimed to develop a multicolor flow cytometry panel to enable detailed phenotypic and functional analysis of distinct macrophage populations during sepsis. The panel was designed to discriminate between classically activated (M1-like) and alternatively activated (M2-like) macrophages, as well as to identify intermediate or transitional phenotypes. Markers were selected based on literature review and preliminary data, with an emphasis on surface proteins involved in macrophage activation, antigen presentation, and cytokine production. The panel was tested using bone marrow samples from a mice CLP (cecal ligation and puncture) model of sepsis. Samples were collected from the femur and cranial bone after 1, 7, 14, and 30 days from the initial operation. Gating strategies validated through FMO controls. Initial results demonstrate a clear separation of macrophage subsets and reveal dynamic shifts in their distribution and activation status throughout sepsis. This panel provides a valuable tool for further studies on macrophage biology in sepsis and may support the identification of novel targets for immunomodulatory therapies.

Keywords: Sepsis, macrophages, immunology, CLP mice

P-56: DXO1 involved in biotic stress response

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Plant stress response is managed by varied and intertwined signaling pathways. One of the mechanisms is changes in RNA metabolism in cells. Such changes are also provided by the DXO1 protein, which is responsible for RNA quality control through its two major functions - decapping of non-canonical NAD+ cap and 5'-3' exonucleolytic activity. The DXO1 response is mainly focused on biotic stress and takes place in the presence of its characteristic N-terminal domain (NTE). Biotic stress signaling induces systemic acquired resistance (SAR) and hyper reactive (HR) response due to fluctuating levels of key factor elements like reactive oxygen species (ROS). The aim of this work was to investigate the importance of functional DXO1 on a plant's ability to combat biotic stressors through different mechanisms. We measured ROS production upon exposure to flagellin in the *dxo1-2* mutant and transgenics *dxo1-2* lines expressing DXO1(WT), DXO1(E394A/D396A) catalytic mutant, DXO1(DN194) lacking the unstructured plant-specific N-terminal domain and DXO1(DN194/E394A/D396A) . Additionally, RT-qPCR was performed to indicate whether there is a correlation between the phenotype and DXO1 mRNA level in the *dxo1-2* mutant. Results indicate a significant role of DXO1 in the processes of ROS scavenging and retention, and uphold that NTE and catalytic domain are crucial in these molecular events. Surprisingly, mRNA of DXO1 doesn't seem to directly influence severance of the *dxo1-2* phenotype, which might suggest a pleiotropic effect affecting observed distinct features.

Keywords: DXO1, RNA metabolism, biotic stress response

P-57: Characterization of Lipid Composition in *Yarrowia lipolytica* Microbial Oil Derived from Alternative Carbon Sources

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The increasing demand for sustainable lipid sources has driven interest in microbial oil production using alternative carbon sources. *Yarrowia lipolytica*, an oleaginous yeast, is a promising candidate for converting waste substrates into valuable lipids. This study investigated the lipid composition of microbial oil produced by *Y. lipolytica* when cultivated in media with waste oil residues and oil extracted from spent coffee grounds as carbon sources. Oil from spent coffee grounds was extracted with n-hexane in Soxhlet apparatus. Waste oil residues were collected from local canteen (waste post-frying mixed oil) and local meet company (lard, post-frying rapeseed oil). Gas Chromatography-Flame Ionization Detection (GC-FID) was used to analyze the fatty acid profile, revealing a monounsaturated and polyunsaturated fatty acids composition. The results highlighted the potential of *Y. lipolytica* for biotechnological applications in biofuel and nutraceutical production, offering an efficient strategy for valorizing industrial and food waste. *Yarrowia lipolytica*, by utilizing a specific waste substrate, produces oil with a composition comparable to that derived from conventional sources, demonstrating its potential for efficient waste bioconversion into valuable lipids. The obtained results will allow for a better understanding of the processes occurring during single-cell oil production and implementing the obtained oil in various industries.

The project is financed from the state budget funds granted by the Minister of Science within the framework of the Programme "Student scientific circles create innovations" (SKN/SP/601167/2024).

Keywords: Lipid composition, GC-FID, waste oil residues, spent coffee grounds, microbial oil, Yarrowia lipolytica

P-58: Refining the role of RIG-I in dsRNA recognition: the impact of 5' end and epitranscriptomic modifications

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Double-stranded RNA (dsRNA) is a key molecular pattern associated with viral infections in human cells. The detection of dsRNA is primarily mediated by the RIG-I-like receptor (RLR) family, which includes RIG-I and MDA5. These receptors play pivotal roles in initiating innate immune responses. RIG-I is specialized in recognizing short dsRNA fragments, whereas MDA5 detects longer dsRNA strands. Upon engagement with their respective dsRNA ligands, both receptors undergo conformational changes that trigger downstream signaling pathways, culminating in the production of type I interferons, pro-inflammatory cytokines, and interferon-stimulated genes (ISGs). The immunogenicity of dsRNA is intricately influenced by modifications at the 5' end and by epitranscriptomic alterations. RIG-I exhibits a pronounced affinity for dsRNA bearing a 5'-triphosphate group, a feature commonly associated with viral genomes. Conversely, the presence of a 5' cap1 structure found on host mRNA can shield dsRNA from RIG-I recognition. Furthermore, specific epitranscriptomic modifications within the dsRNA molecule can modulate its interaction with RLRs. The aim of this study was to further define the role of RIG-I in the recognition of dsRNA using A549 knockout (KO) cell lines lacking RIG-I expression. A panel of dsRNA molecules varying in 5' end and epitranscriptomic modifications was synthesized using in vitro transcription (IVT). The immunogenicity of these dsRNA variants was assessed using a luciferase IFN-β reporter assay. Surprisingly, none of the tested dsRNAs triggered an interferon response in RIG-I-deficient cells. While MDA5 is thought to recognize longer dsRNA duplexes, our data suggest that unmodified cap1-dsRNA exhibits only weak immunogenicity, implying that these transcripts may evade detection by both RIG-I and MDA5. This further underscores the critical function of RIG-I in initiating the innate immune response to viral RNA.

Keywords: double-stranded RNA, RIG-I epitranscriptomic marks, 5' end of dsRNA, innate immunity, viral RNA

P-59: Bone marrow-derived mouse neutrophils as a model for NETs in biomedical studies

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Neutrophils constitute the most abundant cell population in peripheral blood, playing a critical role as the body's first line of defense. These cells, formed in bone marrow (BM), are characterized by a short half-life. Their primary defense mechanisms are phagocytosis, extracellular degranulation, and the formation of neutrophil extracellular traps (NETs) during a process known as NETosis. NET formation involves significant morphological changes in the cell nucleus, resulting in chromatin decondensation, nuclear membrane fragmentation, and the release of network structures containing nuclear and/or mitochondrial DNA along with numerous granule proteins. NETs effectively immobilize and eliminate microorganisms, participating in protective mechanisms. However, simultaneously, it can be responsible for various metabolic diseases such as cancers, diabetes, thrombosis, cardiovascular and autoimmune diseases caused by sterile inflammation induced by activated neutrophils. Moreover, the impact of NETs on the cellular level is not fully elucidated. Therefore, NETs represent an essential research material for analyzing signaling pathway activation and homeostatic disturbances within the organism. Different neutrophil isolation protocols were evaluated to establish an optimal *in vitro* model for assessing the NET influence on the target cells. Therefore bone marrow cell suspensions obtained from mice underwent erythrocyte lysis, followed by density gradient centrifugation using separating compounds Histopaque 1119 and 1077 to isolate the neutrophil fraction. Neutrophils were stimulated with different concentrations of PMA for 3 hours, and then the supernatant was cleared of cellular debris by spinning to isolate NETs. The obtained NET structures were stored at -20°C, and their stability was assessed. Isolated DNA was quantified using a spectrophotometer. These data show BM as a good source of neutrophils for the subsequent acquisition of NETs.

[1] Gao F., Guo Y. (2021) Efficacy and specificity of different methods for human neutrophil extracellular trap isolation and handling. Central European Journal of Immunology 46: 384-387.

Keywords: mouse, neutrophils, NETs

P-60: Application of Morphologically Chiral Gold Nanorods in Plasmonic ELISA for TNFα Detection

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Tumor necrosis factor alpha (TNFα) is a crucial cytokine involved in immune regulation, inflammation, and cell signaling. Its dysregulation is linked to autoimmune disorders, chronic inflammation, and cancer progression, making it a vital biomarker for early disease detection and therapeutic monitoring. However, conventional ELISA assays often lack the sensitivity needed to detect TNFα at physiologically relevant concentrations. This study proposes the use of morphologically chiral gold nanorods in plasmonic ELISA to enhance detection capabilities. These nanostructures undergo an etching process when exposed to oxidized TMB2+, with morphological and optical changes directly correlating to TNFα levels. The study employs UV-Vis spectroscopy, circular dichroism (ECD), and electron microscopy to characterize these changes. This approach offers a highly sensitive and selective method for detecting TNFα, enabling more precise monitoring of inflammatory responses, immune dysregulation, and cancer biomarkers. By integrating nanotechnology with immunoassays, this method could significantly improve early diagnosis and disease management, paving the way for advancements in precision medicine and personalized therapeutic strategies. The ability to detect minimal fluctuations in TNFα levels could lead to earlier interventions, improved treatment outcomes, and a deeper understanding of cytokine-driven pathologies.

Keywords: ELISA, pELISA, nanoparticles, gold nanoparticles, TNF alpha

P-61: Role of clathrin- and caveolin- dependent endocytosis in alphaherpesvirus (EHV-1, HHV-1) entry into murine neuron cells — in vitro studies

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Alphaherpesviruses are a subfamily of herpesviruses, including human herpesvirus type 1 (HHV-1) and equine herpesvirus type 1 (EHV-1). HHV-1 is responsible for cold sores (*herpes labialis*) and nervous system infections, such as herpes simplex encephalitis, particularly in newborns. EHV-1 causes abortions in pregnant mares, neonatal foal mortality, upper respiratory tract infections, and neurological disorders. A hallmark of alphaherpesviruses is their ability to infect nerve cells, where they can establish a latent infection. The primary entry mechanism of alphaherpesviruses into host cells involves the fusion of the viral envelope with the cell membrane. However, some reports suggest these viruses may also utilize alternative entry pathways. Therefore, the present study aimed to investigate the involvement of clathrin- and caveolin-dependent endocytosis in the entry of EHV-1 and HHV-1 into murine neurons cultured *in vitro*. Immunofluorescence assay and confocal microscopy analysis were performed to assess the distribution of clathrin and caveolin proteins in infected neurons and their colocalization with virus antigens. Additionally, the effect of pharmacological inhibitors of clathrin- and caveolin-dependent endocytosis (chlorpromazine and nystatin) on viral entry was assessed using quantitative PCR (qPCR). The findings, together with existing literature, suggest that clathrin- and caveolin-dependent endocytosis may serve as alternative entry pathways for certain alphaherpesvirus strains. This study supports the notion that alphaherpesviruses can exploit multiple entry mechanisms, which may vary depending on cell type and environmental conditions. A comprehensive understanding of these diverse entry strategies could inform the development of novel therapeutic approaches to combat alphaherpesvirus infections in the future.

Keywords: EHV-1, HHV-1, clathrin- and caveolin-dependent endocytosis

P-62: Regulatory Role of the sRNA OmrA in the High-Pathogenicity Island of *Yersinia enterocolitica*

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Yersinia enterocolitica exhibits a dual lifestyle, thriving both as a non-pathogenic saprophyte and as a gastrointestinal enteropathogen. The high virulence of biotype 1B strains is attributed to the presence of the High-Pathogenicity Island (HPI), a genomic region encoding proteins involved in the biosynthesis, regulation, and transport of yersiniabactin—a siderophore essential for iron acquisition. Small regulatory RNAs (sRNAs) play a crucial role in bacterial regulatory networks and stress responses, influencing survival and adaptation. Among them, OmrA is a small regulatory RNA controlled by the EnvZ/OmpR two-component system in response to environmental changes such as osmotic stress and iron limitation. This study aimed to elucidate the role of OmrA sRNA in the regulation of the High-Pathogenicity Island in *Y. enterocolitica*. Experiments were conducted using *Y. enterocolitica* strains with varying OmrA activity. Siderophore production was assessed using the CAS agar diffusion assay, while yersiniabactin levels were quantified via the BacTiter-Glo assay. FyuA protein synthesis was evaluated by Western blot, and gene expression was analyzed using RT-qPCR. Results showed that OmrA stimulates the production of yersiniabactin. Its overexpression enhances FyuA synthesis, the yersiniabactin receptor, both in LB medium and under iron-limited conditions. Additionally, OmrA positively regulates the expression of the HPI-encoded siderophore biosynthesis operon and *fyuA* in response to iron deficiency. These findings highlight the role of OmrA in iron acquisition regulation, although this effect may be indirect. They underscore the importance of OmrA in the pathogenicity and environmental adaptability of *Y. enterocolitica*, suggesting its potential as a target for therapeutic strategies aimed at modulating bacterial behavior and survival.

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Keywords: sRNA, OmrA, Yersinia enterocolitica, HPI, gene expression

P-63: Construction and phenotypic analysis of mutants in pili synthesis-related genes in *Pseudomonas donghuensis* strain P482.

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Plant growth and crop yield are highly influenced by the plant's microbiome, particularly the rhizosphere microbiome. Plant growth promoting rhizobacteria (PGPR) are becoming more widely considered as means of protection against pathogens and yield increase. The focus of our studies is on *Pseudomonas donghuensis* P482, a strain isolated from the rhizosphere of a garden-cultivated tomato (*Lycopersicon esculentum* Mill.) in Gdynia, Poland. *P. donghuensis* P482 shows strong antagonism against plant pathogenic bacteria and fungi, e.g. *Dickeya spp.*, *Pectobacterium spp.*, *P. syringae,* and *Rhizoctonia solani.* It is also an excellent coloniser of plant roots, which is an important trait for its potential use as a PGPR as it promotes niche competition with other bacteria. Plant rhizosphere colonisation and biofilm formation can be influenced by a number of factors, including bacterial motility organelle, such as pili. Pili may play an important role in this phenomenon as they are associated with adhesion to substrates and other cells and are also involved in twitching motility. My project aims to verify whether mutations in genes associated with pili formation in *P. donghuensis* P482 affect the strain's ability to colonise the tomato rhizosphere and form a biofilm. I have generated P482 mutants in genes associated with pili formation. I am investigating the ability of the constructed strains to perform different types of motility, analysing their antagonistic activity, and the ability to form biofilm on abiotic surfaces in minimal media with different carbon sources. I am also using tomato root colonisation assays with the use of the mutant GFP-tagged strains. These studies may provide information on the effects of pili on plant root colonisation and their impact on bacterial function. The results of the study may add to our understanding of the biology of the beneficial *P. donghuensis* P482 strain and its potential use as a plant bioprotectant.

Keywords: Pseudomonas donghuensis P482, pili, biofilm, rhizosphere colonisation

P-64: The influence of elicitation on antioxidant properties of *Polyscias filicifolia* (C. Moore ex E. Fourn.) L. H. Bailey (Araliaceae) callus extracts

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Polyscias filicifolia, commonly known as fern leaf *Panax* is a species of plant which is known in traditional Southeast Asian medicine as an adaptogenic agent. The available data indicate that the ethanolic extracts from *P. filicifolia* callus tissue exhibit many pharmacological properties such as antimicrobial, anti-inflammatory and protein biosynthesis inhibitory activity during hypoxia and myocardial ischemsoli [1].

The study consisted on evaluation of production of phenolic compounds from *P. filicifolia* callus cultivated *in vitro* by applying selected concentration of elicitor - methyl jasmonate (JM), in comparison to tissue that was not treated with it. The callus grow on solid SH medium [2] supplemented with GA_3 1 mg/L with or without JM content under light/dark 12/12 h. Callus tissues treated with JM were collected after 1 week and from control - after 4 weeks. Then, they were lyophilized and extracted. Resulted extracts were investigated for their antioxidant properties including estimation of the content of total phenolics (TPC), total flavonoids (TFC), the potential for scavenging of DPPH radical, the determination of antioxidant power by FRAPP. Extracts with JM were also analysed by Liquid Chromatography-Mass Spectrometry.

The elicitor used in the present study JM, increased the content of TPC, TFC and antioxidant properties in comparison to the control. The potential for radical scavenging of tested extracts was not affected.

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[2] Schenk R., Hildebrandt A. (1972) Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Canadian Journal of Botany 50: 199-204.

Keywords: Polyscias filicifolia, callus extracts, methyl jasmonate, phenolics, flavonoids, potential for radical scavenging, antioxidant properties

P-65: What's new with *Kluyveromyces marxianus*? Exploring its therapeutic potencial

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Probiotic microorganisms inhabit human and animal organisms and help them to function properly. They are naturally present in the digestive tract, skin and other living environments. So far, only individual strains of *Kluyveromyces marxianus* have been studied as probiotics, although they show promise for their health-promoting properties. Significantly, yeast produces EVs (extracellular vescicles), which are filled with proteins, DNA and RNA. Research to date has focused mainly on EVs of pathogenic yeast. Their charge depends on growth conditions, i. e. temperature, culture time, nutrient availability. EVs of *Candida albicans* or *Cryptococcus neoformans* are an essential element for colonization of the human body. However, it should be remembered that the human body is constantly subjected to non-pathogenic microorganisms as well. This is why our research focused on defining new potential yeast probiotics (*K. marxianus* 1 and 2) and characterizing both the morphology and therapeutic potential of their EVs. Probiotics properties taken into account were survival in the digestive system, adhesion to cell line and mucin, antimicrobial activity, hydrophobicity, autoaggregation, antioxidant potential and safety (non-toxicity, no heamolytic and mucinolytic activity). *K. marxianus* B0399 (supplement DiarYeast) was used as a control. By simulating conditions in the human gastrointestinal track, EVs were isolated from culture in SGF (simulated gastric fluid), SIF (simulated small intestinal fluid), SCF (simulated colonic fluid) and SAB as a medium control according to our protocol[1] and characterized by NTA (size and quantity), MS (protein content), TEM (morphology). Moreover, EVs activity on normal and cancer cell lines isolated from the intestine were compared. This research expands our knowledge of probiotic yeasts and their EVs which is limited to this day. Understanding their function could lead to new biotechnological applications in medicine and pharmaceuticals.

This work is part of the project funded by the National Science Centre, Poland (NCN) and carried out under the number 2023/49/B/NZ9/03663.

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Keywords: probiotic yeast, Kluyveromyces marxianus, extracellular vesicles

P-66: Plant tRNA modifying enzyme MIAA influences chloroplasts translation, photosynthesis and cold stress responses

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Chloroplasts genomes encode about 80 proteins, many of which are involved in photosynthesis or gene expression, including all chloroplasts tRNAs. Yet these tRNAs undergo massive chemical modifications catalyzed by different chloroplast-targeted enzymes. The chemical changes introduced into nucleosides are essential for proper decoding of the genetic information and stability of the tRNA structure. Bacteria and plant chloroplasts share the important tRNA modifying enzyme MIAA, catalyzing modification of the adenosine (A) to N^6-isopentenyladenosine (i^6A) at position 37 of the tRNA anticodon. While in *Escherichia coli* loss of MIAA protein results in less efficient translation and increased sensitivity to stress, its significance for plants performance remains unknown. The aim of this study was identification and molecular characterization of mutants in the *MIAA* gene in a model plant *Arabidopsis thaliana* and to verify the hypothesis that the MIAA regulates chloroplast-dependent plant growth.

We show that the plant nuclear-encoded AtMIAA localizes to chloroplasts and seems to conduct similar functions to the bacterial type enzyme. Knock down *miaa* mutants show large transcriptome reprogramming including genes related to photosynthesis, translation, chaperone activity and stress responses, as well disturbed accumulation of PSI and PSII photosynthetic proteins and inhibited growth. Additionally, loss of MIAA function results in a translation - and cold-sensitive phenotypes, as indicated by seedlings chlorosis, decreased photosynthetic efficiency rates and impaired root growth, while germinated on translation inhibitors: lincomycin, chloramphenicol and tetracycline, or grown at 4°C.

This work identifies MIAA as important player controlling chloroplasts and photosynthetic functions during both development and stress growth, probably by promoting organellar translation.

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Keywords: chloroplast, tRNA, MIAA, modification

P-67: Construction of a Titanium-Binding T7 Bacteriophage Using the Phage Display Method

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Phage display is a method based on selecting a polypeptide from a peptide library displayed on the surface of a bacteriophage that binds to a specific target [1]. It is a powerful technique that can be used to attach bacteriophages to various surfaces, including metals [2]. The aim of this study was to modify the genome of the T7 bacteriophage in such a way that the newly displayed polypeptide on its capsid surface would enable binding to a titanium plate. Initially, three polypeptides with the highest affinity for titanium were isolated using a peptide library displayed on the M13 bacteriophage. These sequences were obtained through a biopanning process. Subsequently, they were sequenced and recombined into the T7 phage genome. To confirm their insertion sites, samples were sent for sequencing. The final test involved an input:output assay, which compared the phage titer before and after the biopanning process to identify the most strongly binding polypeptide. Additionally, two previously characterized titanium-binding sequences, TiBP1 and TiBP2, were selected as reference points. As a result of these procedures, T7 bacteriophages displaying titanium-binding peptides were successfully generated. These modified phages exhibited significantly stronger titanium binding than wild-type phages and showed binding efficiency comparable to previously known sequences. Moreover, genome modifications did not negatively affect the phages' ability to infect bacterial cells. These findings present promising prospects for future applications in fields such as implantology.

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[2] Matys S. et al. (2020) Characterization of specifically metal-binding phage clones for selective recovery of cobalt and nickel. Journal of Environmental Chemical Engineering 8: 103606.

Keywords: Phage display, Titanium-binding peptides, Biopanning, Implantology

P-68: Assessing the potential benefits of humic substances extracted from sewage sludge

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Considering the imperative to reduce the use of chemical fertilisers and pesticides, while simultaneously enhancing soil biodiversity, biological methods to improve soil environmental quality are gaining increasing attention. Humic substances (HS) have emerged as valuable soil amendments due to their diverse and beneficial effects on soil health. However, these substances are mainly derived from non-renewable materials such as coal and peat, which are depleting and exacerbating environmental concerns. This underlines the urgency of exploring alternative, renewable sources of HS. A circular approach to wastewater and sludge management offers a resource-efficient strategy that prioritizes the valorisation of waste streams. Recent studies have identified wastewater treatment plants as promising sources of HS, particularly through the recovery of these substances from sludge and reject water. The present research investigates the feasibility of extracting and utilizing HS before and after anaerobic digestion (AD) as potential plant biostimulants, natural pesticides, and soil amendments. Research findings indicate that HS recovered from sewage sludge effectively function as biostimulants for selected plant species, including oats, vetch, garden cress, mustard, and radish, promoting root and stem development. However, no significant stimulatory effects were observed in corn. Interestingly, fractions of HS extracted from sewage sludge before AD have demonstrated antimicrobial properties, effectively reducing the growth of certain phytopathogens such as *B. cinerea* (27-39% reduction) and *S. sclerotiorum* (24-27% reduction). Furthermore, fulvic acids recovered from sewage sludge after AD enhanced the growth of soil microorganisms in liquid culture by up to 144% compared to the control when applied at a concentration of 50 mg/L. Additionally, the application of recovered HS in organic matter-deficient soils improved functional diversity and carbon utilization efficiency in this environment.

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Keywords: extraction, fertilizing potential, humic substances, natural pesticides, sewage sludge

P-69: In search of Cytokinin Homeostasis: The Interplay of CKX2 and PUP7 in Rye

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Phytohormones are crucial for proper cereal development. Cytokinin (CK) as part of the plant signalling pathway play a significant role in stimulating cell division. Besides its primal organ formation purpose, they are also involved in stress responses, seed settling and development. Accordingly genes related to cytokinin metabolism can be crucial to increase yield. In case of ensuring homeostasis the *CKX2* and *PUP7* genes are working reversely. In rice *CKX2* is a member of the *CKX* gene family that encode oxidase/dehydrogenases, which are responsible for degradation. Active form of cytokinin are irreversibly inactivate by cleaving the N6-side chain, which can be visible in reduced tiller number and grain number per plant. On the other hand *PUP7*, member of purine permease family, is responsible for transport of CK. This allows the interaction between phytohormones and receptors at the target site. Potentially resulting in increased grain. Both of these genes can be identified in common rye (*Secale cereale* L.) However its function and expression profile in polish agriculture remain uncertain. Rye is a significant pillar of Polish agriculture, and is cultivated on more than 700. 000 hectares. It is important to study rye genetics related to grain size to optimizing the yield-cost ratio. In this study, we conduct research of *CKX2* and *PUP7* expression level 7, 14 and 21 days after pollen. We made comparison of expression and morphometric data of various rye genotypes. Research materials were obtained from the Secale cereale collection from Polish Gene Bank and Polish Breeders. Obtained results indicate that *CKX2* and *PUP7* shows expression in every genotype of tested rye.

This research was part of R-Grain project funded by the National Centre for Research and Development, grant number LIDER/32/0170/L-12/20/NCBR/2021.

Keywords: Rye, Cytokinin, CKX2, PUP7, expression, grain size

P-70: Barcoding Droplet Composition in Droplet Screen-seq: A New Platform for Ultra-High Throughput Microbial Consortia Analysis

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Current methods for creating and screening microbial consortia are limited in scalability and are unable to comprehensively link input conditions to microbial outcomes, as the indexing methods create a major bottleneck in throughput. To address this issue, the Droplet Screen-seq method is being developed. It introduces a codomer-based DNA indexing system that encodes and tracks millions of unique microdroplet compositions, enabling the systematic creation and screening of complex microbial consortia on a microfluidic Lab-on-a-Chip device.

The Droplet Screen-seq platform involves the use of microfluidic chips fabricated by soft lithography. To validate the DNA codomers under experimental conditions, the chips were used to create millions of mock microbial consortia in 100 picoliter droplets, by trapping sets of standard bacteria in droplets. Each input composition was encoded with a specific combination of codomers, that were optimized for the longest lifetime and monitored through qPCR.

In our research, we successfully developed and optimized a functional codomer-based method for encoding and detecting the input composition of millions of droplets. We have also designed and validated the functionality of microfluidic chips for combining up to 5 codomer-tagged input solutions, and picoinjecting droplets with lysis buffers and PCR mixes. Ongoing development efforts focus on barcoding of codomers and 16S rDNA and integrating multiple components into a single workflow.

The results obtained to date form a promising foundation for the future development of the Screen-seq platform. The codomer detection will be paired with 16S rDNA sequencing and ultra-high throughput biocatalytic assays in droplets. The Screen-seq platform will be validated by screening consortia from environmental samples (involving fluorescence-activated droplet sorting) for the discovery of antibiotic-producing bacteria. We believe that the codomer indexing method will be relevant for several applications in the Screen-seq method.

Project funded and supported by National Science Centre Project ID: SONATA BIS 2023/50/E/ST4/00545

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Keywords: droplet microfluidics, microbial consortia, next-generation sequencing, ultra high-throughput screening

P-71: Gamma-decalactone as an active compund in edible packaging films

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Gamma-decalactone as a fragrance substance is produced by the β-oxidation process of ricinolenic acid, which was produced by hydrolysis of castor oil. This compound has a flavoring character with an oily-peach aroma, which is commonly used in food and cosmetics. An attractive way to produce aromatic compounds is the production of gamma-decalactone by biotransformation of castor oil using microorganisms, such as *Candida*, *Rhodotorula*, *Sporidiobolus*, *Pichia* oraz *Yarrowia*. The work aimed to investigate the effect of gamma-decalactone as active compound on the antimicrobial activity, microstructure, optical and mechanical properties of pectin packaging films. The work included the preparation of film-forming solutions from apple pectin at a concentration of 5% with the addition of glycerol as a plasticizer to which gamma-decalactone was added at concentrations of 2. 5, 5 and 10%. The solutions were poured into Petri dishes of constant volume and dried at 50C for 24 h. The obtained films were tested for antimicrobial activity, optical (colour, opacity and UV-VIS light transmission), mechanical properties (tensile strength, elongation at break, Young's modulus) and structural (Fourier-transform infrared spectroscopy and scanning electron microscopy). The addition of gamma-decalactone into pectin films showed antimicrobial activity againts selected moulds and bacteria, modified colour and higher UV light barrier, reduced the breaking strength and Young's modulus while causing an increase in relative elongation, but only at concentrations of 5 and 10 ml/100 ml. Fourier transform infrared spectroscopy analysis confirmed the presence of typical functional groups in control pectin films and pure gamma-decalactone. However, in the case of combining gamma-decalactone with a pectin matrix, functional groups occurring in pectin were observed, suggesting hydrolysis of this compound. The addition of gamma-decalactone significantly affected the structure of the film surface, which was more uneven and porous.

Keywords: gamma-decalactone, edible films, active packaging, apple pectin

P-72: Development and characterization of a highly stable FGF2 variants and their role in cell signaling, migration and glucose uptake

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FGF2 belongs to the canonical fibroblast growth factors. This protein is important for proper limb development, lung branching morphogenesis, chondrogenesis, neuronal differentiation, myogenesis and angiogenesis. It also participates in regenerative processes of bone and skin tissue. However, the stability of FGF2 and its susceptibility to proteolytic degradation is a major obstacle to future therapeutic applications. Therefore, the aim of our work was to obtain a variants of FGF2 protein with increased thermodynamical stability and resistance to proteolysis. We designed 40 single mutations to affect the thermodynamic stability of the protein and 8 substitutions that reduce heparin affinity to improve the pharmacokinetics of FGF2. All mutant variants were overproduced in a bacterial system and purified. We then determined their thermodynamic parameters by thermal denaturation analysis. The most stable single variants were verified for proteolytic resistance and their ability to activate FGFR-dependent signaling pathways. The mutations most affecting denaturation temperature and resistance to degradation were used to construct multiple mutants and multiple variants with reduced affinity for heparin. The most stable multiple variant had a denaturation temperature more than 27. 6°C degrees higher than the wild type. We characterized the most promising variants in terms of their proliferative properties, receptor affinity, signaling kinetics and susceptibility to proteolysis. The results confirmed that the introduced mutations did not disrupt FGF2 structure or its interaction with the receptor. The two most stable variants retained biological activity even after 5 days of incubation at 70°C, while the wild type was completely inactive. In conclusion, we obtained stable FGF2 variants characterized by significant resistance to degradation and extended lifetimes, which have high potential for use in therapeutic applications in the future.

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Keywords: Fibroblast Growth Factor, FGF, FGF2, stability, signaling

P-73: Cell line-specific metabolic adaptations in glioblastoma: LDH as a marker in 2D and 3D cultures

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Glioblastoma as a highly aggressive brain tumor has a significant metabolic heterogeneity across different cell lines and microenvironments such as normoxia and hypoxia. The key enzyme in anaerobic metabolism - lactate dehydrogenase (LDH) - is also a marker of cell damage, making it useful for exploring metabolic adaptations. The aim of the study is to examine how different glioblastoma cell lines respond to radiation, different environments and 3D culture conditions through LDH assays. Experiments were conducted using glioblastoma LN229, U87, U251 and GL261 in 2D and 3D models under normoxic (21% O2) and hypoxic (1% O2) conditions. To examine the influence of radiotherapy, 2D models were subjected to 1-6 Gy of X-ray irradiation. The LDH assay (LDH-Glo Cytotoxicity Assay, Promega) was performed 48h and 120h after radiation in 2D models and after 5 days of normoxic and hypoxic incubation in 3D spheroids. No significant differences observed between normoxia and hypoxia in 2D cultures. LDH levels also remained unchanged between 48h and 120h. In 3D models, LDH levels were 55% higher in normoxic LN229 than in normoxic U87 and 67% higher than in normoxic GL261. Hypoxic U87 released 60% more LDH than normoxic U87. Reduced ATP levels were exhibited in the hypoxic environment - 56% lower in LN229, 51% in U87, 35% in U251 and 25% in GL261 compared to cell cultures in normoxia. Hypoxia altered the response of glioblastoma cells. Differences in LDH levels released by cells suggest the influence of radiation, environmental conditions and cell line-specific properties on glioblastoma multiforme metabolism adaptation. Larger variation of data in 3D models show the impact of cell packing and internal necrosis in individual spheroids. The results indicate that tracking LDH levels can be used in understanding glioblastoma cells metabolism shifts depending on various conditions.

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Keywords: glioblastoma, LDH assay, metabolic heterogeneity, X-ray irradiation, normoxia, hypoxia, spheroid model

P-74: Identifying new effector genes of the mitochondrial retrograde pathway in Candida albicans

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Retrograde regulation (RTG) is broadly defined as cellular responses to changes in the functional state of mitochondria. We aimed to study the RTG pathway in *Candida albicans*, which in addition to being an opportunistic human pathogen is also separated from *Saccharomyces cerevisiae* by two evolutionarily important events, making it an interesting research model. The RTG pathway in *S. cerevisiae* is activated by the translocation of the ScRtg1/Rtg3 dimer to the nucleus, but the dimer is retained in the cytoplasm when the pathway is inactive. In *C. albicans*, orthologous CaRtg1/Rtg3 dimer is constitutively localised to the nucleus, therefore the mechanism of activation is presumably different from *S. cerevisiae*. The same applies to the known effector genes of the RTG pathway in those two organisms - in *S. cerevisiae* the pathway mainly controls genes encoding enzymes of the tricarboxylate acid and glyoxylate cycles, whilst in *C. albicans* only 4 effectors involved in galactose metabolism and alternative respiration are known (*CaGAL1*, *CaGAL7*, *CaGAL10* and *CaAOX2*). We expect other targets of this pathway, that are yet to be identified, to be involved in the survival of *C. albicans*' cells in unfavourable conditions. The aim of our project was to identify new targets of the RTG pathway in *C. albicans*. We performed chromatin immunoprecipitation sequencing (ChIP-seq) analysis on respiratory deficient strains and selected the most promising genes, on whose promoters we performed electrophoretic mobility shift assays (EMSA). We will present the results of the bioinformatic enrichment analyses of the ChIP-seq hits and confirmation of new effector genes using EMSA.

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Keywords: Candida albicans, mitochondrial retrograde pathway, RTG pathway, ChIP-seq

P-75: AI-supported image analysis of droplet deformation for high-throughput and label-free measurement of microbial proteolytic activity.

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Introduction: Droplet-based screening is a dynamic area of modern microfluidics, used for functional screening and selection of bacterial strains [1]. By leveraging differences in the shape of flowing droplet depending on gelatin concentration, the YOLOv11s-seg machine learning algorithm [2] was used to detect and identify droplets containing proteolytic bacteria that degrade gelatin.

Experimental: Machine learning was applied to analyze footage from flowing droplets with microcultures containing proteolytic bacterial strains. Initially, individual proteolytic bacterial cells were encapsulated in picoliter droplets. Following incubation at 40°C, the droplets were reintroduced into detection microfluidic devices, where they were deformed at a flow- focusing junction by perpendicular oil streams. The extent of deformation was directly proportional to the concentration of gelatin within the droplets. AI-based image analysis was then employed to detect and correlate proteolytic activity using a pre-established calibration curve. This approach enabled the detection and sorting of positive droplets containing proteolytic microcolonies, identified by their highest degree of deformation. The video was processed using the program ffmpeg to extract individual frames at a rate of two frames per second. The frames were then selected, analyzed, and saved in a format suitable for training the YOLOv11s-seg algorithm. The model was subsequently trained over 100 epochs, and its performance was evaluated.

Results: The encapsulation and cultivation of proteolytic strains were conducted in 100 pL droplets, enabling millions of droplets to be processed in a single experiment. Various flow rates for squeezing oils and video recording settings were tested to determine and select the optimal conditions. The performance analysis of the YOLOv11-seg model demonstrated a high detection accuracy higher than 99% in identifying positive droplets with low gelatin content due to the proteolytic activity of bacteria. The model achieved a proces.

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[2] Khanam R., Hussain M. (2024) YOLOv11: An Overview of the Key Architectural Enhancements. arXiv:2410.17725 [cs.CV].

Keywords: Computer Vision, label-free sorter, proteolityc activity

P-76: Identification of a pathogenic variant of COL1A1 in a 10-years-old donor diagnosed with OI type I

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Collagen Type I (col I) is a major component of connective tissues such as the dermis, bones, tendons, and ligaments. It plays a key role in tissue remodeling during repair and wound healing. Col I is a heterotrimer made of two pro-α1 chains and one pro-α2 chain, encoded by the *COL1A1* and *COL1A2* genes. Reduced or abnormal Col I production, due to mutations in these genes, leads to *osteogenesis imperfecta* (*OI*), a group of genetic disorders causing bone fragility. The aim of this study was to analyse the genetic background and expression of several extracellular matrix (ECM)-related genes in a 10-year-old male with severe clinical symptoms of *OI* type I. Skin biopsies were collected from the patient with *OI* type I and a 13-year-old healthy male, as a control. DNA was isolated from primary skin fibroblast cultures for sequencing and polymorphism analysis. We also examined the expression of 14 ECM-related genes. Our findings revealed a single nucleotide variation (SNV) mutation in the *COL1A1* gene (g.11030G>C), resulting in a glycine-to-alanine substitution, alongside several polymorphic variants in *COL1A1* (rs3840870 - 4bp and rs1061237 - MnlI) and *COL1A2* (rs412777- PvuII and rs42524) genes. Gene expression analysis showed an increased expression of *COL1A1* and *COL3A1,* along with reduced expression of *COL1A2*, *ECM1, HSPA1A*, and *HSPA1B* in fibroblasts from the *OI* patient, compared to the control. In conclusion, genetic analysis revealed a *COL1A1* mutation (g.11030G>C)-typically associated with milder *OI* phenotypes. However, the patient's relatively severe clinical symptoms were likely influenced by additional polymorphisms detected in *COL1A1* (rs3840870, rs1061237) and *COL1A2* (rs42524, rs412777). This combination of molecular factors may contribute to the more severe clinical presentation in this case. We believe that our findings represent an important step toward understanding the impact of DNA sequence variations and their expression on the clinical course of *OI*.

This work was supported by KWN-2-O09/N/8/N awarded to M.B.

Keywords: COL1A1, COL1A2, Osteogenesis imperfecta, mutation, skin fibroblasts

P-77: Bismuth(III) complexes of 1,2,4,5-tetrasubstituted imidazoles as promising antimicrobial and urease-inhibitory agents

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Bismuth(III) compounds have been widely used for many years, for example, bismuth subsalicylate in gastrointestinal treatments, bismuth subnitrate and subgallate as antiseptics and wound-healing agents, and bismuth subcitrate for the eradication of *Helicobacter pylori* infections. However, their low solubility limits bioavailability and therapeutic potential. In this study, novel Bi(III) complexes with 1,2,4,5-tetrasubstituted imidazole ligands were synthesized and characterized using physicochemical methods. The biological evaluation demonstrated that these complexes exhibit significantly enhanced antibacterial activity compared to their parent ligands and commercial Bi(III) subcitrate (De-Nol). The most potent complexes showed minimum inhibitory concentrations in the range of 0.061-0.086 mmol/mL against *Staphylococcus saprophyticus*, *Pseudomonas putida* and *Proteus vulgaris* which are associated with hospital-acquired infections, urinary tract disorders, and antibiotic resistance, posing significant challenges in clinical treatment. Strong urease inhibition was observed, with inhibition concentrations as low as 0.031 mmol/mL, surpassing the reference drug Bi(III) subcitrate (De-Nol). Since urease increases environmental pH and facilitates bacterial colonization, its inhibition can disrupt bacterial survival mechanisms and enhance susceptibility to antimicrobial treatment. Molecular docking studies revealed strong binding affinities of the synthesized compounds not only to urease but also to key bacterial enzymes involved in metabolism and virulence, including MurE ligase, DNA gyrase, and metal-binding proteins. These interactions suggest multiple potential mechanisms of antimicrobial action, further supporting the therapeutic potential of the Bi(III) complexes. These findings suggest that Bi(III) imidazole complexes could serve as promising candidates for biomedical applications targeting bacterial infections and urease-associated pathologies.

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Keywords: Bismuth(III) complexes, antimicrobial activity, urease inhibition, molecular docking

P-78: Analysis of prokaryotic symbionts among eglenozoan protists

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Introduction: Symbiosis with prokaryotes has played a crucial role in the evolution of eukaryotes. Even though protists represent the majority of the eukaryotic diveristy, for many lineages their prokaryotic symbionts are still unknown. One such lineage, Euglenozoa, is ecologically important in aquatic ecosystems, yet their symbiotic relations with prokaryotes still remain largely unknown.

Methods: Single cells, manually picked form both cultures and environmental samples were starved overnight and lysed by several freeze-thaw cycles. Released DNA was amplified using REPLI-g Single Cell Kit. Partial sequences of 16S/18S rRNA genes were amplified using the polymerase chain reaction method. The amplicons were then sequenced by Sanger method. Some of the amplicons were sequenced by Illumina NovaSeq platform and Oxford Nanopore MinION for better reads quality. Lastly bioinformatic analysis was performed in order to align and trim the sequences and construct the phylogenetic tree.

Results: Over 40 different culture strains and over 70 environmental cell samples were analysed using the above method. Among class *Euglenida* a prokaryotic group of symbionts was identified, unoficially named „*Euglenibacteraceae"* which belongs to class *Gammaproteobacteria.* The presence of prokaryotic symbiont was detected in above 10% of analysed cells.

Conclusions: The results show that the sybiotic relationships between euglenozoans and prokaryotes are present, especially among highly diverse class of *Euglenida*. While the exact role of this relationship still remanis to be investigated, its presence should not be overlooked. Analyses of aquatic environmental DNA could now better describe studied communities and potential interactions between euglenozoans and prokaryotes.

Keywords: Euglenozoa, Endosymbiosis, Prokaryotic symbionts, Euglenida, Protists

P-79: Transcriptomic and physiological responses of *Umbelopsis* to endohyphal bacteria — *Paraburkholderia*

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Fungi and bacteria are known to live together across diverse environments, and their interactions are crucial to maintain the balance and function of terrestrial ecosystems. One specific form of such interaction involves bacteria that live inside fungal hyphae, known as endohyphal bacteria (EHB). These intimate relationships are important for the survival and adaptation of both organisms, showcasing the intricate nature of microbial partnerships. Although *Mucoromycota* fungi have long been recognized as hosts for endohyphal bacteria, it wasn't until 2021 that *Umbelopsis* species were also confirmed to carry endosymbiotic bacteria, primarily from the *Paraburkholderia* genus.

In our research, we examine how *Paraburkholderia* influence the growth, fitness, and stress response of their fungal host *Umbelopsis* (WA50703 strain). To evaluate growth dynamics, we cultured *Umbelopsis* strains either containing (EHB+) or lacking (EHB-) endobacteria on a minimal nutrient medium. Fungal biomass was collected daily over a two-week period, then dried and weighed to generate growth curves. Our findings suggest that under stress conditions, the presence of endohyphal bacteria may reduce the growth rate of the fungal host.

To further investigate how these bacterial symbionts affect the overall fitness of *Umbelopsis*, we conducted a transcriptomic analysis comparing EHB+ and EHB- strains under stressors like high temperature and osmotic stress. RNA was extracted using Trizol reagent, then treated with DNase, purified, and used to construct cDNA libraries. These libraries were sequenced on an Illumina HiSeq platform, followed by bioinformatic and statistical analysis to identify genes with differential expression. The outcomes of this study provide valuable insights into how bacterial symbionts influence the physiology and stress adaptation of their fungal hosts.

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Keywords: fungi, endohyphal bacteria, RNAseq, transcriptoms

P-80: Fed-Batch Cultivation of *Yarrowia lipolytica*: Lipid Production and Supernatant Valorization

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The oleaginous yeast *Yarrowia lipolytica* has gained considerable attention as a microbial cell factory for sustainable lipid and protein production. In this study, fed-batch cultivation strategies were employed to enhance lipid accumulation and biomass yield through controlled nutrient feeding at distinct intervals. Among the tested regimes, a 24-hour feeding frequency yielded the highest lipid concentration (11.22 g/L), while a 6-6-12 hour feeding schedule resulted in peak protein content (43.75% dry mass). Detailed fatty acid profiling revealed high proportions of unsaturated fatty acids, with oleic acid as the dominant component, supporting the suitability of the lipids for nutraceutical or oleochemical applications. Beyond primary metabolite production, this work places significant emphasis on the valorization of the post-fermentation supernatant. The freeze-dried supernatant demonstrated a calorific value of 10.43 kJ/g, indicating its potential as a biofuel precursor. Additionally, elevated levels of phosphorus (430 mg/kg) and potassium (428 mg/kg) suggest promising applicability in biofertilizer or biostimulant formulations. These findings propose a holistic bioprocessing approach that integrates microbial oil production with downstream waste stream utilization, contributing to the development of circular bioeconomy models in industrial biotechnology.

Keywords: Yarrowia lipolytica, Fed-batch cultivation, Microbial oil, Oleaginous yeast

P-81: Unveiling the secrets of stroma-leukemia communication and its pro-leukemic outcomes: focus on the CD44 protein

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Interactions between leukemia cells and their microenvironment play a vital role in the development of drug resistance. We have previously shown [1] that contact-dependent, tunnelling nanotube-mediated transfer of vesicles from bone marrow stromal cells to chronic myeloid leukemia (CML) results in protection of CML cells from imatinib-induced apoptosis. Our trans-SILAC studies have shown that proteins associated with cancer progression are transported towards leukemia along with stromal vesicles. Among them we identified CD44, protein associated with drug resistance and metastasis. Presented studies aimed to confirm the CD44 transfer from stroma to leukemia and elucidate its role in acceptor cells. Human bone marrow stromal and leukemic cells were co-cultured to study interactions within leukemia microenvironment. Confocal microscopy and FACS were used to assess the level and transfer of CD44. To identify differences in the transcriptomes of leukemic cells that received or did not receive CD44 from stroma, we performed bulk RNA-seq analysis on RNA samples from sorted cells. To check invasive and migratory properties, CD44+ and CD44- leukemic cells were sorted followed by trans-well experiments. Fluorescently tagged stromal CD44 protein was detected within tunneling nanotubes and on the leukemic cell membranes. The presence of CD44 protein in CML cells was associated with increased multidrug resistance and enhanced invasiveness of CD44+ cells. These observations were supported by transcriptome analyses, as cells that acquired CD44 from stroma exhibited higher expression of genes related to ECM reorganization. We present a novel mechanism of microenvironment-mediated protection in which CD44 is directly transferred from stroma to leukemia cells, leading to drug resistance and increased invasiveness. Observed mechanisms may represent a potential target for future therapeutic strategies aimed at inhibiting leukemic cell-stroma interactions and overcoming drug resistance.

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[1] Kolba M. et al. (2019) Tunneling nanotube-mediated intercellular vesicle and protein transfer in the stroma-provided imatinib resistance in chronic myeloid leukemia cells. Cell Death & Disease 10: 817.

Keywords: leukemia, bone marrow microenvironment, tunneling nanotubes, cell adhesion, resistance

P-82: *Rickettsia* diversity and prevalence in *Dermacentor reticulatus* ticks from two expansion zones in Poland

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Dermacentor reticulatus ticks are significant vectors of infectious diseases, including rickettsioses - a group of zoonoses caused by bacteria of the genus *Rickettsia*. Rickettsioses, such as spotted fever, pose a growing health concern for humans and animals due to their potentially severe course and increasing incidence in Europe. In Poland, the dynamic expansion of this tick species has been observed, potentially influencing the epidemiology of tick-borne pathogens. This study aimed to compare the prevalence and species diversity of *Rickettsia* bacteria in *D. reticulatus* populations from two expansion regions: eastern and western Poland. Ticks collected between 2023 and 2024 were analyzed using molecular methods, including PCR with primers specific for the *gltA* and *ompB* genes. Additionally, droplet digital PCR was used for precise quantification of *Rickettsia* DNA, while sequencing and phylogenetic analysis of specific gene fragments determined species diversity and genetic relationships. The prevalence of *Rickettsia* bacteria was higher in ticks from the western expansion region (17.23%) compared to the eastern region (2.7%). In both regions, *Rickettsia raoultii* was the dominant species; however, in the western region, additional species such as *R. monacensis* and *R. helvetica* were identified, indicating greater species diversity. The higher infection rate in the western region may result from environmental differences, host diversity, and distinct expansion dynamics. The greater *Rickettsia* diversity suggests a potentially higher risk of rickettsial disease transmission in this area. These findings highlight the need for continued monitoring of tick-borne pathogens, as the western population, currently undergoing dynamic expansion, may play a more significant role in rickettsiosis epidemiology.

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Keywords: Rickettsia, rickettsioses, Dermacentor reticulatus, tick-borne diseases, epidemiology

P-83: ChatGPT in Higher Education — A Learning Aid or an Obstacle?

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The rapid advancement of Large Language Models (LLMs), such as ChatGPT, has sparked debates regarding their role in higher education. While these AI tools offer students assistance in information retrieval, summarization, and even content generation, concerns arise about their impact on **critical thinking, learning effort, and academic integrity**. This study examines the cognitive and behavioral effects of using ChatGPT among Polish university students. We analyze how reliance on LLMs influences **mental effort, information retention, and problem-solving skills** across different academic disciplines. Through surveys and controlled experiments, we compare students who actively integrate AI assistance with those who engage in traditional study methods. The findings highlight differences in **knowledge absorption, creativity, and engagement levels**, addressing whether AI serves as a **learning enhancer or an intellectual crutch**. Furthermore, we discuss the **long-term educational implications** of widespread AI adoption, including potential shifts in **academic assessment, personalized learning, and student autonomy**. The study aims to provide insights for educators and policymakers on integrating AI-driven tools responsibly while preserving essential cognitive skills in higher education.

This research was conducted independently and did not receive any external funding. However, I acknowledge the guidance of my academic supervisors and peers who contributed valuable insights to this study.

Keywords: ChatGPT, AI in education, student learning, mental effort, academic integrity, cognitive impact

P-84: Synthesis of the m7GpppG-AuNP Conjugate and Investigation of Its Ability to Bind eIF4E Protein Using Gel Electrophoresis

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A key role in the function of mRNA is played by the cap structure present at its 5'-end. It consists of a 7-methylguanosine linked to the first nucleotide of the transcript by a 5'-5' triphosphate bond. The cap enables the transcript to be recognized by the cell's translational machinery through binding with the eIF4E protein, a key translation initiator. Overexpression and dysregulation of eIF4E are linked to cancer processes, making it a promising target for therapies aimed at inhibiting excessive translational activity. The function of eIF4E is closely dependent on its interaction with the cap, so synthetic analogs of this structure are potential therapeutics capable of blocking the protein's binding to mRNA, leading to translation inhibition. The challenge remains the efficient delivery of cap analogs into cells. Under physiological conditions, these molecules carry a negative charge, which limits their ability to cross cell membranes. A promising solution to this problem is the use of gold nanoparticles, which, due to their large surface area, enable stable ligand binding and can act as carriers for therapeutic molecules.

The purpose of the present study was to synthesize a cap structure analog capable of functionalizing the surface of gold nanoparticles, to form a conjugate of the obtained analog with the nanoparticles, and to use electrophoretic methods to qualitatively evaluate the binding of the eIF4E protein to the prepared conjugate. In this study, a cap analog in the form of m^7GpppG^S was successfully synthesized and used to modify the surface of gold nanoparticles. The interaction of the eIF4E protein with the modified nanoparticles was analyzed using polyacrylamide and agarose gel electrophoresis methods. In both cases, after incubation of the modified nanoparticles with the protein, a change in their electrophoretic mobility was observed compared to control samples, suggesting an interaction between the proposed conjugate and the eIF4E protein.

[1] Shanmugasundaram M., Senthilvelan A., Kore A. (2022) Recent Advances in Modified Cap Analogs: Synthesis, Biochemical Properties, and mRNA Based Vaccines. The Chemical Record 22: e202200005.
[2] Kurpiejewski K. et al. (2025) Gold nanoparticles functionalized with mRNA cap analogs as a strategy to inhibit eIF4E in cancer therapy. Journal of Drug Delivery Science and Technology 107: 106820.

Keywords: mRNA cap analog, gold nanoparticles, eIF4E, gel electrophoresis

P-85: No lysines, no problem? How FBXL15 breaks the rules of ubiquitination

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The ubiquitin-proteasome system (UPS) plays a pivotal role in protein degradation. In the canonical pathway, this system targets proteins for proteasome-dependent degradation by attaching ubiquitin molecules to specific lysine residues in these proteins. This study explores the concept of non-canonical, i. e. lysine-independent, proteasome-dependent ubiquitination in the context of lysine deserts - regions within proteins markedly deficient in lysine residues - and their potential to deter ubiquitin-dependent proteolysis. Our research focuses on the human FBXL15 (F-box and leucine rich repeat protein 15), which functions as a receptor in the cullin-RING E3 ubiquitin ligase (CRL) complex. FBXL15, by directing SMURF1 (SMAD specific E3 ubiquitin protein ligase 1) degradation, regulates BMP (bone morphogenetic protein) signalling during embryonic development and adult bone formation. The main aim of the study was to elucidate the effect of lysine deserts on the ubiquitination and turnover of FBXL15, which was predicted to have an unstable C-terminus. Utilising a HiBiT tagging strategy (Promega) in human HEK293 cells the study revealed that there is no significant difference in protein turnover between wild-type FBXL15 and its mutant lacking the sole two lysines, demonstrating the role of lysine desert in protecting this receptor from missense canonical ubiquitination from the hands of the E3 ligase complex. Furthermore, disruption of lysine deserts by various arginine-to-lysine triple-point mutations does not alter the stability of FBXL15 compared to the wild type, suggesting a possible high resistance to ubiquitin-dependent degradation. Our study deepens understanding of lysine deserts in the ubiquitin-proteasome system, revealing how they help prevent unwanted ubiquitination. These insights highlight key regulatory mechanisms that safeguard protein turnover and impact physiological processes like BMP signaling and bone mass maintenance.

This research was supported by the National Science Centre, Poland (grant SONATABIS number 2021/42/E/NZ1/00190 to W.P.).

Keywords: ubiquitin-proteasome system, lysine deserts, FBXL15, protein turnover and stability

P-86: Synthesis and biological evaluation of TBBi derivatives as potential CK2 inhibitors with anticancer properties

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Protein kinase CK2 is a constitutively active serine/threonine kinase that plays a critical role in regulating key cellular processes such as proliferation, apoptosis and tumour progression, making it a promising target for anticancer therapy. Previous studies have shown that 4,5,6,7-tetrabromo-1*H*-benzimidazole derivatives exhibit significant anticancer activity through CK2 inhibition [1]. Based on these findings, we synthesised a series of novel 4,5,6,7-tetrabromo-1*H*-benzimidazole-2-thiol derivatives functionalized at *S* or *N* atom. Our approach involved *S*-alkylation of 4,5,6,7-tetrabromo-1*H*-benzimidazole-2-thiol or *N*-alkylation of 4,5,6,7-tetrabromo-2-(methylsulfanyl)-1*H*-benzimidazole (K37) using various alkylating agents (e. g. phenacyl halides, chloroacetone, methyl iodide). All reactions took place with satisfactory yields ranging from 50% to 85%. The synthesized compounds were evaluated for recombinant CK2 inhibition using the ADP-Glo™ Kinase Assay, based on the measurement of luminescence. Additionally, the MTT-based cytotoxicity studies were conducted against four cancer cell lines, i.e. MCF-7, MDA-MB-231, CCRF-CEM and K-562, representing hormone dependent breast cancer, triple negative breast cancer, acute lymphoblastic leukemia and chronic myeloid leukemia, respectively. Our findings indicate that some of the new derivatives more efficiently inhibited the viability of the studied cell lines than the parent compounds. The preliminary results suggest that the applied structural modifications influence cytotoxicity of the tested compounds, highlighting their potential as new promising anticancer agents for further investigation.

This research was supported by the Warsaw University of Technology within the Excellence Initiative: Research University (IDUB) program.

[1] Łukowska-Chojnacka E. et al. (2024) Synthesis and evaluation of anticancer activity of new 4,5,6,7-tetrabromo-1H-benzimidazole derivatives. Bioorganic Chemistry 153: 107880.

Keywords: 4,5,6,7-Tetrabromo-1H-benzimidazole, Antitumor activity, Cytotoxic activity, Protein kinase CK2

P-87: Study of WHIRLY1 protein turnover in *Arabidopsis thaliana*

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WHIRLY (WHY) are best known for binding single-stranded DNA. Most plants have WHY1 and WHY2 proteins, but *Arabidopsis thaliana* also has additional isoform - WHY3. The WHIRLYs are considered as multitargeted proteins possibly localized in chloroplasts, mitochondria and/or nucleus. That phenomenon arises a question about how the WHIRLY proteins turnover is regulated? Its regulation most likely involves post-translational modifications or interactions with other proteins. To reveal potential ways of WHIRLY1 turnover, we decided to investigate the role of outer chloroplast membrane SP1 E3 ligase. SP1 belongs to SP protein family, and together with TOC (*translocon on the outer chloroplast membrane*) complexes, and SP2 proteins is a part of the chloroplast-associated protein degradation system (CHLORAD). It is a proteolytic system that removes chloroplast proteins. SP1 ligase is involved in the ubiquitination of TOC proteins and thus indirectly regulate chloroplast biogenesis and also affect the transport of chloroplast proteins. Therefore, we hypothesized that SP1 may be involved also in WHY1 regulation. Our cell free degradation assay showed that in *sp1* protein extract, half-life of GST-WHY1 is extended in comparison to WT protein extract. This suggests that SP1 may influence WHY1 turnover. Moreover, inside chloroplast there is a set of protein proteases including Deg proteases family, responsible for maintaining proper protein homeostasis. We suspected that due to the identical localization of both proteins in chloroplasts, it is possible that the WHY1 protein is degraded by proteolysis by the Deg2 protein inside the chloroplasts. We confirmed interactions between WHY1 and Deg2 by BiFC method, what in turn strongly suggests that Deg2 can perform WHY1 proteolysis. Our results shows two potential ways for WHIRLY1 protein degradation, by proteasome 26S and by chloroplast protease Deg2. These findings significantly enhance our understanding of WHIRLY regulation mechanisms.

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Keywords: WHIRLY, SP1, Deg2, CHLORAD

P-88: Impact of T cell activation on lysosomal function during inflammation

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T cells are central mediators of immune responses, yet the impact of their activation on lysosomal function during inflammation remains incompletely understood. In this study, we explored how T cell activity influences the autophagy-lysosomal pathway (ALP), using both the Jurkat T cell line and primary $CD4^+$ T lymphocytes isolated from healthy donors. Acidic organelle content was quantified *via* spectrophotometry and flow cytometry, revealing a marked increase in acidic compartments following activation. Individual components of ALP were characterized by confocal and transmission electron microscopy, demonstrating structural remodeling and increased organelle abundance. A comprehensive transcriptional profile of lysosome-related genes in activated Jurkat cells revealed upregulation of almost all of 90 key genes involved in lysosomal biogenesis and function. Importantly, we extended our analysis to $CD4^+$ T cell subpopulations (Th1, Th2, Th17, Th22), quantifying protein levels of lysosome-related markers and identifying subtype-specific expression patterns. These findings are particularly relevant in the context of autoimmune and inflammatory diseases, where imbalances between T helper subsets contribute to pathogenesis. Altogether, our data highlight a dynamic interplay between T cell activation and lysosomal function, providing novel insights into how intracellular organelle modulation may shape immune responses and suggesting potential avenues for therapeutic intervention in immune-mediated disorders.

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Keywords: T cell, lysosome, lysosomal biogenesis, autophagy

P-89: Study of the influence of selected substrate parameters on the SERS effect

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Surface-enhanced Raman spectroscopy is a technique with a great range of applications, due to the Raman effect providing characteristic spectra, containing structural and more in-depth information, on varied molecules. The SERS substrates can be designed for specific use, to be more biocompatible — e.g., gold-based, modified /w graphene etc. The method is undergoing a renaissance, thanks to the ongoing miniaturization process, as well as many proposals of new hybrid substrate types, combining the best of both worlds from plasmonic and non-plasmonic materials. The endeavour of progress requires further investigation of the basic mechanism standing behind the SERS, which is not fully understood. Optimization has a better chance of success if it's led by mechanistic study, not only trial and error. The poster is a proposal of such a study using controlled surface structures to visualize SERS signal with SEM imaging, and correlate structure types with signal strength and possibly a mechanism. Substrates provided by Dr Piotr Wróbel from the Department of Physics, University of Warsaw, are made by colloidal lithography and physical vapour deposition. The matrix of polystyrene nanospheres is covered by 1 nm of germanium — serving as a wetting agent, and varied widths of silver and gold layers. The end-goal substrate should be covered by separated nanospheres — weak hot spots (weak localized surface plasmon resonance LSPR), and around them due to the shadowing effect should develop a nanogap — a strong hot spot (strong LSPR). Germanium wetting layer makes sure that the rest of the surface is ultra-flat, and will not exhibit LSPR (electromagnetic mechanism), only chemical mechanism. Future substrates will be optimized for the separation of electromagnetic and chemical effects. The research so far was mostly done by hand correlation of SEM images and high-resolution Raman maps. Some results were acquired by simultaneous Raman mapping and SEM imaging using the Renishaw InLux.

Keywords: SERS, plasmonic substrates, hybrid substrates, chemical mechanism, electromagnetic mechanism, LSPR

P-90: Development of bacterial cellulose-PLA composites via 3D printing and in situ biosynthesis

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In recent years, biocomposites have gained significant attention as environmentally friendly alternatives to traditional synthetic materials due to their biodegradability and sustainability. Although several studies have explored the production of polylactide (PLA)-bacterial cellulose (BC) composites, they have been largely limited to combining pre-fabricated biopolymers using various physical or chemical methods. The aim of this study was to develop an innovative and facile method for the fabrication of PLA-BC composites, incorporating 3D printing technology. PLA scaffolds were designed using CAD software and fabricated via fused filament fabrication (FFF). The printed scaffolds were conditioned with chemical and physical agents prior to cultivation to prevent undesired bacterial contamination. They were then placed in vessels containing Hestrin-Schramm (H-S) medium inoculated with *Komagataeibacter xylinus*. Bacterial cellulose was allowed to grow directly on the PLA scaffolds over several days until the desired thickness was achieved. Following cultivation, the PLA-BC composites were purified and stored in hydrated form at low temperature. The materials were characterized using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) to assess their morphology and physicochemical properties. The developed composites exhibit strong potential for applications in biotechnology, particularly as biocarriers for immobilized biocatalysts.

Keywords: bacterial cellulose, polylactide, 3D print, biopolymers

P-91: Phenytoin reduces hypersensitivity by altering microglia/macrophage activity at the spinal cord level in a rat model of neuropathic pain

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Neuropathic pain, a chronic condition resulting from nervous system damage, affects millions worldwide and remains a significant clinical challenge. Phenytoin, a sodium channel blocker, has gained attention for its potential analgesic properties beyond epilepsy treatment. However, its mechanism of action in neuropathy remains to be fully elucidated. The aim of the study was to evaluate the antinociceptive effects of phenytoin in a rat model of chronic constriction injury (CCI) of the sciatic nerve (Bennett model of neuropathic pain) and its influence on IBA-1 (a macrophage/microglia marker) and CD44 (a macrophage/lymphocyte marker) in the dorsal root ganglia (DRGs) and/or spinal cord. The experiments were conducted on naïve and CCI-exposed male Wistar rats. Animals received intraperitoneal injections of vehicle or phenytoin (30 mg/kg) 16 and 1 h before CCI and then once daily for 7 days following CCI. The mechanical and thermal hypersensitivity was assessed 2 and 7 days after CCI using von Frey and cold plate tests, respectively. The protein levels of IBA-1 and CD44 were measured by western blot analyses on DRGs and/or spinal cord tissues 7 days after CCI. Phenytoin significantly reduced hypersensitivity to mechanical and thermal stimuli, indicating its significant antinociceptive properties. Western blot analysis revealed that CCI increased IBA-1 levels in the DRGs and spinal cord; notably phenytoin treatment significantly diminished observed changes. In turn, CCI led to a reduction in CD44 levels in DRGs, which was not affected by phenytoin administration. These findings suggest that the analgesic effects of phenytoin in neuropathic pain may be related to suppressed microglial/macrophage activation and infiltration in the spinal cord. Further studies are necessary to fully understand the neuroimmune mechanisms involved in the analgesic effects of phenytoin in neuropathy.

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Keywords: phenytoin, neuropathic pain, IBA-1, CD44, microglia, macrophages

P-92: The influence of selected material properties of bacterial cellulose membranes on the permeability of inorganic salt compounds.

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Bacterial cellulose (BC) is a polysaccharide synthesized by various microorganisms, with the most well-known and frequently used being *Komagataeibacter xylinus* (*K. xylinus*). This biopolymer is distinguished by its high chemical purity, water retention capacity, and porosity, making it an attractive material for biomedical applications. The aim of this study was to analyze the impact of selected material properties of bacterial cellulose membranes on the permeability of low-molecular-weight inorganic salts. BC biosynthesis was carried out under static culture conditions in 6-well plates, using the *K. xylinus* strain (ATCC 53524) in Herstrin-Schramm liquid medium. The BC culture was incubated for 3, 5, and 10 days, and then purified in 0. 1 M NaOH. The physicochemical characteristics were analyzed using ATR-FTIR, identifying the characteristic chemical bonds of the biopolymer. The permeability of BC membranes for NaCl solutions (150 mM and 75 mM) was tested in a modified Franz cell, consisting of a controller, conductometers, and donor and acceptor chambers. The results showed that the dry mass yield of BC increased with the extension of the culture time, reaching a maximum after 3 days of culture, while the highest wet mass yield was recorded after 5 days of culture. FTIR spectroscopy revealed changes in the structure of BC, indicating a decrease in crystallinity and a reduction in the content of the alpha form (Iα) of BC with the extension of the culture time. The permeability of the membranes was assessed based on the k constant and effective permeability (Peff). Regardless of the NaCl concentration, the highest permeability was recorded for the 3-day membrane, and the lowest for the 10-day membrane, which may suggest progressive structural reorganization and reduction in porosity. The results indicate that the structure of BC plays a key role in its permeability, making it a promising material for applications requiring efficient gas and/or liquid transport, such as wound dressings or filtration materials.

Keywords: bacterial cellulose, permeability, small-molecule compounds, ATR-FTIR

P-93: IN SILICO EXPRESSION OF HUMAN PROTEIN DYRK1A IN *PSEUDOALTEROMONAS HALOPLANKTIS* 125

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DYRK1A and CDKL5 are critical kinases for neurodevelopment, and their deficiency has been linked to severe neurological disorders including intellectual disability and epilepsy. DYRK1A and CDKL5 share significant structural similarities, particularly their extensive intrinsically disordered regions (IDR). It was recently demonstrated that the TAT peptide was applied to facilitate CDKL5 delivery across the blood-brain barrier, making a suitable candidate for therapeutic use. CDKL5-TAT was already successfully expressed in *Pseudomonas haloplanktis TAC125*, a psychrophilic bacterium, but not efficiently in E. coli. This study examines whether DYRK1A-TAT can be successfully expressed in *P. haloplanktis* TAC125 by optimizing its sequence and expression method in silico. To optimize DYRK1A-TAT expression in *P. haloplanktis TAC125*, we employed a variety of computational tools such as AlphaFold, SoluProt, ProtParam, HADDOCK, OPTIMIZER. Our results indicate that predicted DYRK1A-TAT structure is stable with minimal conformational disruptions. Codon optimization of *P. haloplanktis* TAC125 predicts optimal translational efficiency and docking shows no interference by TAT with the catalytic domain of DYRK1A. These findings collectively suggest that *P. haloplanktis TAC125* can be a valid host for DYRK1A-TAT expression and must be experimentally validated. The optimized construct possesses promising qualities like structural stability and solubility, essential for efficient expression and purification. These in silico observations provide a strong foundation for experimental validation in the future, with potential applications in the development of novel protein-based therapeutics for neurological disorders linked to DYRK1A deficiency.

Keywords: heterologous expression, DYRK1A, TAC125

P-94: Development of a new Rabies virus with changed tropism

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Vision is the fundamental sense that underpins life quality. According to the World Health Organization, over 2. 2 billion people worldwide suffer from visual impairment or blindness The prevalence of these conditions requires the development of new treatments. Gene and/or viral therapies represent a recent development in the treatment of degenerative retinal diseases. One unconventional solution under investigation is the use Rabies virus (RV), a (-)RNA virus known for its ability to infect neurons via retrograde transport (from postsynaptic to presynaptic cells). Using RV it is possible to obtain a high and rapid expression of multiple genes. However, a crucial challenge in gene delivery is achieving specificity, as RV infections are not cell-type unique. To specifically control gene delivery, G-deleted RV(RVΔG) will be pseudotyped with a chimeric protein responsible for interacting with the cell-type-specific receptor. We hypothesize that RV pseudotyped with the extracellular domain of LRIT3 (RV-exLRIT3) will infect ON-bipolar cells specifically. As a preliminary step, we generated a lentivirus encoding the exLRIT3-oG protein (the glycoprotein of the Rabies virus). This virus was then used to transduce BHK-21 cells, thereby establishing a stable cell line. The following stage involved the production of the pseudotyped Rabies virus (RV-exLRIT3), which was injected into the eyes of mice (wild type and mouse model of retinal degeneration) and examined by immunohistochemistry assays. This study aims to enhance understanding of pseudotyping strategies and investigate their application in gene therapies, with the objective of developing novel approaches for the treatment of retinal degenerative diseases.

National Science Center, Poland (2019/34/E/NZ5/00434), National Science Center, Poland (2022/47/B/NZ5/03023)

Keywords: Rabies virus, pseudotyping, lentivirus, gene therapy

P-95: Development of a novel microfluidic approach for identifying and tracking horizontal gene transfer events

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Nowadays, pathogenic, antibiotic-resistant microbes, antibiotic resistance genes (ARGs) and virulence genes (VGs) are recognized as novel types of emerging (biological) contaminants. This rapidly escalating global threat poses severe risks to public health, agriculture and environmental stability. Addressing this problem requires a combination of various molecular and high-throughput methods. The development of a novel microfluidics platform provides a promising pathway for targeted profiling of selected genes in single bacterial cells with improved scalability, speed and efficiency. For setting this method, we used a microfluidic Lab-On-Chip device to encapsulate single *E. coli* cells into picoliter droplets together with lysis buffer and beads with immobilized barcoded primers for detection of both 16S rDNA and ARGs at a single cell level. Next, droplets with single cell lysates were pico-injected at high throughput with PCR mix and TaqMan probes complementary to the targeted ARGs. Then emulsion PCR was executed and positive droplets were distinguished thanks toflorescent signal from probes complementary to genes of interests. Droplets serving as isolated microbioreactors, allowed carrying out parallel multiplex PCR to profile bacterial cells. Each positive droplet contained a single cell lysate with amplicons of both types of genes (16S rDNA and targeted ARGs). The set of primers and probes were designed and optimized to detect multiple ARGs in a single experiment. Identification of the specific ARG is possible thanks tomeasurements of fluorescence light using both microscopy and high-throughput detection in a microfluidic device with a frequency of up to 2 thousand droplets per second. On-chip fluorescence-based sorting will allow to select "positive droplets", in which various ARGs were amplified. Future applications of this method combined with amplicon-based NGS can link selected genes with their hosts and enable targeted single-bacteria genome profiling to track horizontal gene transfer events.

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Keywords: Droplet microfluidic, high-throughput, ARGs, HGT, single-bacteria genom profiling, DNA barcoding

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