Book of Abstracts

16th Biotechnology Students’ Conference

BOOK of ABSTRACTS

1-2 June 2026, Gdańsk

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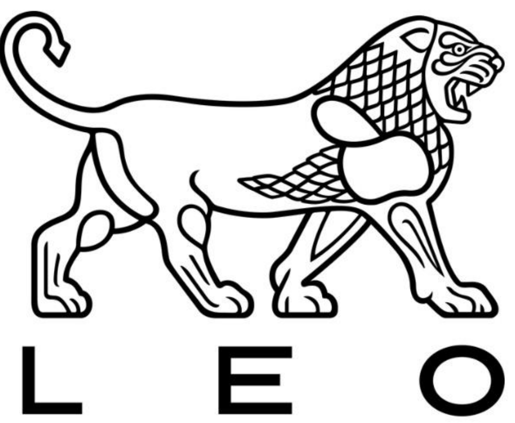
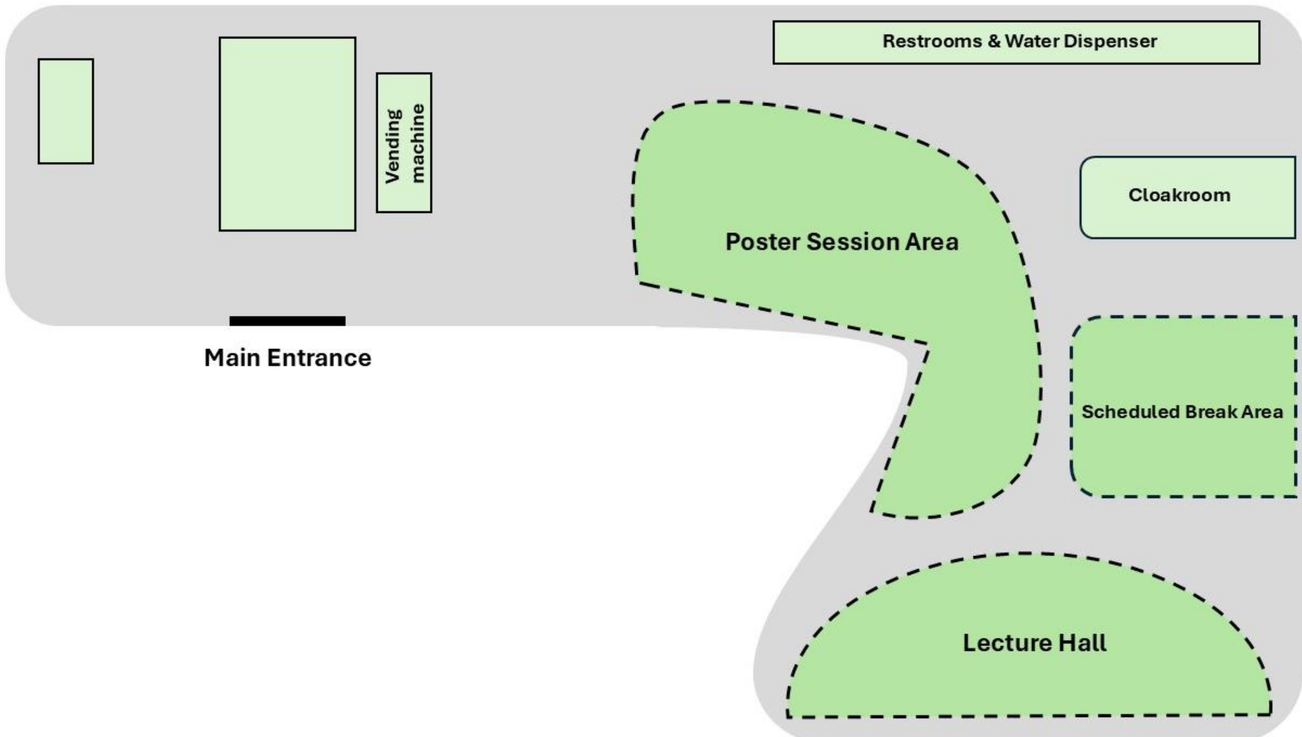


Conference map



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Agenda Overview

DAY 1 MONDAY, 1st JUNE 2026

9:00 - 9:10 **INAUGURATION CEREMONY**

9:10 - 9:30 **PLENARY LECTURE**
dr Sylwia Klińska-Bąchor

9:30 - 10:30 **ORAL SESSION #1**

10:30 - 10:50 **SCHEDULED BREAK**

10:50 - 11:50 **ORAL SESSION #2**

11:50 - 12:50 **POSTER SESSION**

12:50 - 13:10 **SCHEDULED BREAK**

13:10 - 14:10 **ORAL SESSION #3**

14:10 - 14:30 **SCHEDULED BREAK**

14:30 - 15:40 **ORAL SESSION #4**

DAY 2 TUESDAY, 2nd JUNE 2026

9:00 - 10:00 **ORAL SESSION #5**

10:00 - 10:20 **SCHEDULED BREAK**

10:20 - 11:40 **ORAL SESSION #6**

11:40 - 12:00 **SCHEDULED BREAK**

12:00 - 13:30 **ORAL SESSION #7**

13:30 - 13:50 **SCHEDULED BREAK**

13:50 - 14:50 **ORAL SESSION #8**

14:50 - 15:20 **SCHEDULED BREAK**

15:20 - 16:20 **AWARD CEREMONY**

Conference Program

DAY 1 MONDAY, 1st JUNE 2026

9:00 - 9:10 INAUGURATION CEREMONY

9:10 - 9:30 PLENARY LECTURE

PL Fantastic acyltransferases and how to use them: a story of plant stress and omega-3 fatty acids

Sylwia Klińska-Bąchor, PhD, Laboratory of Plany Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG

9:30 - 10:30 ORAL SESSION #1

O.1 Identification of *Ochrobactrum anthropi* ATCC 49188 genes essential for biofilm formation and attachment to biotic surfaces

Ziemowit Juszczuk, Laboratory of Plant Microbiology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.2 Screening of the *Pseudomonas donghuensis* P482 miniTn5 mutant library for its ability to form biofilms on abiotic surfaces and plant tissues

Ewa Śpiewak, Laboratory of Plant Microbiology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.3 Effect of culture media on nitrosative stress sensitivity of *Pseudomonas donghuensis* P482 and characterization of the produced pigment

Jan Jezierski, Laboratory of Plant Microbiology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.4 Biochemical characterization of enzymes involved in the synthesis of wax esters and fatty acid ethyl esters in microsomal fractions from Arabidopsis thaliana leaves

Alicja Czyż, Laboratory of Plant Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

O.5 The role of phospholipid:diacylglycerol acyltransferase1 (PDAT1) in plant adaptation to high temperature stress

Karolina Słowińska, Laboratory of Plant Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

O.6 The influence of drought stress on the development of Arabidopsis plants with varied expression of acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT) encoding genes

Maksymilian Patoła, Laboratory of Plant Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

10:30 - 10:50 SCHEDULED BREAK

10:50 - 11:50 ORAL SESSION #2

O.7 Optimization of recombinant West Nile Virus NS2B-NS3 protease production in a bacterial gene expression system

Karina Franchuk, Laboratory of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology UG&MUG.

O.8 Development of a potential mRNA vaccine against infectious Bronchitis Virus in chickens

Zuzanna Derwich, Laboratory of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology UG&MUG.

O.9 The FGFR signaling in regulation of DNA damage response

Michalina Wilkowska, Laboratory of Molecular Enzymology and Oncology.

O.10 The role of YAP/TAZ in developing resistance to anti-ER therapy in luminal breast cancer cells

Olga Gniewkowska, Laboratory of Molecular Enzymology and Oncology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.11 The significance of regulatory interactions between the FGFR and HER2 pathways in the progression of luminal breast cancer

Maja Siedlecka, Laboratory of Molecular Enzymology and Oncology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.12 The role of FGFR4 - Aurora A – RAD51 interaction in the response of triple-negative breast cancer cells to anti-cancer drugs

Wiktoria Rysz, Laboratory of Molecular Enzymology and Oncology, Intercollegiate Faculty of Biotechnology UG&MUG.

11:50 - 12:50 POSTER SESSION

12:50 - 13:10 SCHEDULED BREAK

13:10 - 14:10 ORAL SESSION #3

O.13 Preparation of a research model for the analysis of the occurrence of the plasmid DNA phase separation phenomenon in bacteria

Adam Borys, Laboratory of Molecular Biology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.14 Construction of vectors for analysis of the intracellular localization of Clp proteases

Małgorzata Mazur, Laboratory of Molecular Biology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.15 Extension of the plugin functionality for running molecular dynamics simulations in the PyMOL program

Katarzyna Wirzba, Laboratory of Biomolecular Systems Simulations, Intercollegiate Faculty of Biotechnology UG&MUG.

O.16 Structural and functional analysis of a potentially pathogenicubiquitin variant encoded by the human UBC gene

Maja Wawrzonowska, Laboratory of Protein Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

O.17 The study of Hsp70-substrate complex initiated by Class B J-domain proteins

Sofía Gómez García, Department of Protein Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

O.18 Identification of protein variants associated with salivary gland tumors

Marta Studzienna, Laboratory of Biopolymers Structure, Intercollegiate Faculty of Biotechnology UG&MUG.

14:10 -14:30 SCHEDULED BREAK

14:30 - 15:40 ORAL SESSION #4

O.19 Optimization of the parameters of the chosen chromatographic step to ensure the optimal purity and quality of the obtained protein

Oskar Osiński, Laboratory of Virus Molecular Biology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.20 Optimization of selected cultivation parameters for a genetically modified CHO cell line in order to achieve the desired product quality after the fermentation stage

Rafał Rosiek, Laboratory of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology UG&MUG.

O.21 Evaluation of the effect of soil extracts on bacteria causing blackleg and soft rot diseases in potato

Maksymilian Frąckowiak, Laboratory of Physical Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

O.22 Development, optimization and validation of a GC-MS method for the quantitative analysis of short-chain fatty acids (SCFA) as markers of metabolic activity of the human gut microbiome

Olga Kuchcińska, Laboratory of Physical Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

O.23 Biological properties of *Paenibacillus polymyxa* against *Dickeya spp*. and *Pectobacterium spp*. and genomic analysis of selected strains

Rozalia Kornelia Rębarz, Laboratory of Physical Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

O.24 Interaction of platinum nanoparticles with idarubicin

Aliaksandra Yurchak, Laboratory of Biophysics, Intercollegiate Faculty of Biotechnology UG&MUG.

O.25 Interactions of titanium nanoparticles with doxorubicin and cisplatin

Aleksander Kowalewski, Laboratory of Biophysics, Intercollegiate Faculty of Biotechnology UG&MUG.

DAY 2 TUESDAY, 2nd JUNE 2026

9:00 - 10:00 ORAL SESSION #5

O.26 Investigation of the role of auxins in the interaction between *Arabidopsis thaliana* and *Pectobacterium betavasculorum*

Ewelina Janiak, Laboratory of Plant Protection and Biotechnology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.27 The effect of phage-encoded transcription factors of prophage phi027 on the orientation of the flagellar switch and flagellum biogenesis in *Clostridioides difficile*

Grzegorz Jabłoński, Division of Molecular Bacteriology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.28 Recombinant Bacillus subtilis spores as a weapon against *Porphyromonas gingivalis*

Kacper Cisło, Division of Molecular Bacteriology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.29 Mapping of transcription factor Xre binding sites

Jan Hodór, Laboratory of Molecular Bacteriology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.30 Characteristics of integrase of bacteriophages infecting *Clostridioides difficile*

Marta Drąg, Laboratory of Molecular Bacteriology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.31 Social interactions between *Dickeya solani* and *Bacillus subtilis*

Zuzanna Dobrowolska, Laboratory of Molecular Bacteriology, Intercollegiate Faculty of Biotechnology UG&MUG.

10:00 - 10:20 SCHEDULED BREAK

10:20 - 11:40 ORAL SESSION #6

O.32 *Drosera zigzagia* extracts towards *Galleria mellonella* and their antimicrobial activity against *Vibrio spp.*

Magdalena Kosmała, Laboratory of Biologically Active Compounds, Intercollegiate Faculty of Biotechnology UG&MUG.

O.33 The influence of *Solanum dulcamara* exposure to chemical contaminants from the group of perfluoroalkyl compounds in the *in vitro* model

Natalia Tołoczko, Laboratory of Biologically Active Compounds, Intercollegiate Faculty of Biotechnology UG&MUG.

O.34 Assessment of the synergistic activity of silver ions combined with quinone against clinical isolates of *Pseudomonas aeruginosa* with varied antibiotic resistance profiles in planktonic and biofilm cultures

Kacper Dąbkowski, Laboratory of Biologically Active Compounds, Intercollegiate Faculty of Biotechnology UG&MUG.

O.35 Enterotoxin C gene expression in the interaction of *Staphylococcus aureus* with keratinocytes

Sonia Gendera, Laboratory of Photobiology and Molecular Diagnostics, Intercollegiate Faculty of Biotechnology UG&MUG.

O.36 The effects of green light irradiation on the A549 human non-small cell lung cancer cell line

Natalia Buć, Laboratory of Photobiology and Molecular Diagnostics, Intercollegiate Faculty of Biotechnology UG&MUG.

O.37 The effects of red light on neural stem cell proliferation and differentiation *in vitro* under normal and pathological conditions

Karol Jankowski, Laboratory of Photobiology and Molecular Diagnostics, Intercollegiate Faculty of Biotechnology UG&MUG.

O.38 Investigation of the effect of photodynamic inactivation on the transfer of drug resistance genes via conjugation in *Escherichia coli*

Anna Milewska, Laboratory of Photobiology and Molecular Diagnostics, Intercollegiate Faculty of Biotechnology UG&MUG.

O.39 Sublethal antimicrobial photodynamic inactivation modulates efflux pump activity in *Escherichia coli*

Amelia Kusiak, Laboratory of Photobiology and Molecular Diagnostics, Intercollegiate Faculty of Biotechnology UG&MUG.

11:40 - 12:00 SCHEDULED BREAK

12:00 - 13:30 ORAL SESSION #7

O.40 The role of CD73 in the regulation of angiogenesis through lipid metabolism

Luiza Malińska, Laboratory of Cell Biology and Immunology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.41 Selection of laboratory assays for reliable assessment of complement-dependent cytotoxicity

Nadia Panasiuk, Laboratory of Cell Biology and Immunology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.42 Modulation of the complement system classical pathway activity by variants of C2 protein

Anna Solecka, Department of Cell Biology and Immunology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.43 Development of a GASDALIE Fc-Engineered Anti-C4d Monoclonal Antibody to Overcome Tumor Resistance via Enhanced ADCC

Julian Zakrzewski, Laboratory of Cell Biology and Immunology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.44 The role of the β-hairpin region of DNAJA2 in preventing Aβ42 aggregation

Jan Edward Skurski, Laboratory of Evolutionary Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

O.45 *In vitro* analysis of the interaction between the bacterial IscU protein and the Hsp70 system specialized in the iron-sulfur cluster biogenesis

Kornelia Kaim, Laboratory of Evolutionary Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

O.46 Measurement of DNAJB1 affinity for the N-terminal region of amyloid-β and crystallisation of the DNAJB1-Aβ complex

Ignacy Renczyński, Laboratory of Evolutionary Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

O.47 A Unique N-Terminal Domain Enables Hairpin Unwinding by Efa DNA Polymerase

Weronika Łuszczewska, Laboratory of Structural Biology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.48 Purification of the ubiquitination machinery components essential for studying the interaction between Salmonella and the host ubiquitin–proteasome system

Roksana Bohdasheva, Laboratory of Structural Biology, Intercollegiate Faculty of Biotechnology UG&MUG.

13:30 - 13:50 SCHEDULED BREAK

13:50 - 14:50 ORAL SESSION #8

O.49 Analysis of changes occurring in tumour-educated platelets (TEPs) as a result of interactions with cells from selected cancer cell lines

Marlena Cinkusz, Department of Translational Oncology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.50 Optimization of the protocol for detection of circulating tumor cells in breast cancer patients

Wiktor Grudniewski, Division of Translational Oncology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.51 Validation of a nanopore sequencing-based method for genotyping hereditary alpha-tryptasemia (HαT) in patients with Hymenoptera venom anaphylaxis

Weronika Wojdacz, Department of Molecular Biology of Viruses, Intercollegiate Faculty of Biotechnology UG&MUG.

O.52 Identification of amino acids of the Human cytomegalovirus IE1 protein responsible for chromosome binding

Mateusz Galiński, Laboratory of Virus Molecular Biology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.53 Investigation of immunomodulatory properties of SARS-CoV-2 ORF3d miniprotein

Paulina Pałkowska, Laboratory of Virus Molecular Biology, Intercollegiate Faculty of Biotechnology UG&MUG.

O.54 Characterization of recombinant SARS-CoV-2 spike proteins expressed in *Leishmania tarentolae*

Aleksandra Jankiewicz, Laboratory of Virus Molecular Biology, Intercollegiate Faculty of Biotechnology UG&MUG.

14:50 - 15:20 SCHEDULED BREAK

15:20 - 16:20 AWARD CEREMONY

PL – Fantastic acyltransferases and how to use them: a story of plant stress and omega-3 fatty acids

Sylwia Klińska-Bąchor, PhD
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Laboratory of Plant Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

Acyltransferases are enzymes that catalyze the transfer of acyl groups (fatty acids) and play a crucial role in the biosynthesis and remodeling of membrane and storage lipids. Due to their central role in lipid metabolism, these enzymes are major players in plant adaptation to adverse environmental conditions and have significant biotechnological potential.

This lecture addresses the role of selected plant acyltransferases in regulating plant responses to environmental stresses induced by extreme temperatures. The focus is on enzymes involved in the biosynthesis of membrane phospholipids, such as phosphatidylcholine and phosphatidylethanolamine, as well as triacylglycerols, the major storage lipids in plants. This knowledge provides a basis for the potential application of these enzymes in developing stress-resilient crop varieties. The study involves both the model plant *Arabidopsis thaliana* and the oilseed crop *Camelina sativa*.

In addition, the presentation focuses on the role of acyltransferases in the biosynthesis of omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs). Attention is given to microalgal acyltransferases, as microalgae are natural producers of these compounds. The presented research focuses on acyltransferases involved in the biosynthesis of eicosapentaenoic acid (EPA), one of the major omega-3 LC-PUFAs and in improving the omega-3 to omega-6 fatty acid ratio. This work involves acyltransferases derived from *Phaeodactylum tricornutum*, a marine diatom, which are expressed in yeast systems for biochemical characterization and in plants, where metabolic engineering approaches are applied to enable sustainable omega-3 production.

Funding: National Science Centre, Poland projects no: 2017/25/B/NZ3/00721; 2018/30/Q/NZ3/00497; 2023/51/B/NZ3/00253. NSFC China projects no: 41776175 and 31961133008. Foundation for Polish Science (FNP); scholarship START 2025.

O.1 – Identification of *Ochrobactrum anthropi* ATCC 49188 genes essential for biofilm formation and attachment to biotic surfaces

Ziemowit Juszczuk

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Under the supervision of Magdalena Rajewska, PhD of the Laboratory of Plant Microbiology, Intercollegiate Faculty of Biotechnology UG&MUG.

Ochrobactrum anthropi is a cosmopolitan species widely distributed across various environments, commonly isolated from plant rhizosphere, soil or water. In clinical settings, the bacterium is recognized as an opportunistic pathogen responsible for nosocomial infections (e.g., catheter-associated). Its identification is problematic because of extreme similarity in genetic sequence with *Brucella* spp. On the other hand, *O. anthropi* constitutively produces AHL-degrading enzymes (AHL-signal molecules) resulting in Quorum Sensing disruption of phytopathogens such as *Pectobacterium* spp. or *Dickeya* spp. Furthermore, its ability to form biofilms facilitates the colonization of host plants.

In this study, transposon mutagenesis was used to generate transposon mutants of *O. anthropi*. This approach allows for the identification of new genes with new features. The obtained library of mutants was evaluated for their biofilm formation capacity on abiotic surface (polystyrene) and adhesion efficiency to tomato roots (*Solanum lycopersicum*). Mutants exhibiting a reduction in biofilm formation up to 90% compared to the wild-type strain were selected for their ability to attach to biotic surface. To quantify adhesion to biotic surface, attachment assays were conducted using tomato seedlings. Colonization intensity of seedlings inoculated with the mutants were analysed by isolating bacteria from tomato roots.

Understanding the mechanisms of biofilm formation is of critical importance; from a medical perspective, it may facilitate the future development of novel strategies to eradicate antibiotic-resistant bacteria from hospital environments, while from an agricultural perspective, it offers the potential to enhance crop yields by exploiting QQ mechanisms against phytopathogenic bacteria. widely distributed across various environments, commonly isolated from plant rhizosphere, soil or water. In clinical settings, the bacterium is recognized as an opportunistic pathogen responsible for nosocomial infections (e.g., catheter-associated). Its identification is problematic because of extreme similarity in genetic sequence with *Brucella* spp. On the other hand, *O. anthropi* constitutively produces AHL-degrading enzymes (AHL-signal molecules) resulting in Quorum Sensing disruption of phytopathogens such as *Pectobacterium* spp. or *Dickeya* spp. Furthermore, its ability to form biofilms facilitates the colonization of host plants.

O.2 – Screening of the *Pseudomonas donghuensis* P482 miniTn5 mutant library for its ability to form biofilms on abiotic surfaces and plant tissues

Ewa Śpiewak
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Under the supervision of Magdalena Rajewska, PhD of the Laboratory of Plant Microbiology, Intercollegiate Faculty of Biotechnology UG&MUG.

Plant Growth-Promoting Rhizobacteria (PGPR), the beneficial bacteria inhabiting rhizosphere, protect plants against pathogens and/or improve plant growth and crop yield through various mechanisms. The application of PGPR in agriculture, as biocontrol agents, may potentially reduce the use of artificial fertilizers and chemical agents, which contribute to environmental pollution. However, a better understanding of the biology and physiology of these beneficial strains is still needed. *Pseudomonas donghuensis* P482, an isolate from tomato rhizosphere, is a potential biocontrol agent due to its strong antagonistic activity against bacterial and fungal plant pathogens such as *Pectobacterium* spp., *Dickeya* spp., or *Rhizoctonia solani*. P482 efficiently colonizes tomato, maize and potato rhizosphere which is important in competition with pathogens for ecological niche. When colonizing plant roots bacteria form biofilms on the tissue surface what enhances their survival and beneficial effect on the host. Despite a link between plant tissue colonization and biofilm formation these processes may depend on different external and internal factors. Earlier research on P482 showed that biofilm formation depends on the available carbon source and type of surface, and that mutants affected in biofilm formation on abiotic substratum were still able to successfully colonize plant rhizosphere. Therefore, the genetic determinants of root colonization in this strain still need to be elucidated.

In this study *P. donghuensis* P482 miniTn5 mutant library was screened to identify mutants with altered biofilm formation and/or colonization ability. First, the ability to form biofilm in the presence of different carbon sources on polystyrene using crystal violet staining and exopolysaccharide production by Congo Red assay were analyzed. Then, for selected mutants, attachment to tomato roots was tested and mutants impaired in adhesion to roots were chosen for further characterization. Their motility and ability to form biofilm on glass under previously tested nutritional conditions were analyzed. As well as their colonization of tomato rhizosphere in long-term assay. Mutants were also sequenced to determine the site of miniTn5 transposition.

Screening of P482 transposon mutant library identified mutants with altered biofilm formation and exopolysaccharide production. Biofilm formation ability of P482 WT and its mutants was dependent on type of abiotic substratum and nutritional conditions. Among six mutants, tested in attachment assay, three showed significant differences in adhesion to tomato roots but only one colonized tomato rhizosphere less efficiently than P482 WT. Sequencing of selected mutants revealed genes potentially involved in processes mentioned above. The results of this study may expand our knowledge about beneficial *P. donghuensis* P482 strain and its molecular mechanisms underlying biofilm formation on abiotic surfaces and plant tissues.

O.3 – Effect of culture media on nitrosative stress sensitivity of *Pseudomonas donghuensis* P482 and characterization of the produced pigment

Jan Jezierski

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Under the supervision of Dorota M. Krzyżanowska, PhD, DSc of the Laboratory of Plant Microbiology, Intercollegiate Faculty of Biotechnology UG&MUG.

Nitric oxide (NO) plays an important role in mammals, plants, and microorganisms. Its functions are diverse, ranging from regulation of blood pressure, through mediation of plant responses to various biotic and abiotic stresses, to modulation of bacterial biofilm formation. NO and its derivatives, collectively known as reactive nitrogen species (RNS), can also induce nitrosative stress, damaging cellular components and potentially leading to cell death.

Although the role of NO has been well established in the symbiosis between rhizobia and leguminous plants, much less is known about its function in other plant-associated bacteria. Our previous studies demonstrated that exudates from tomato roots alter the transcriptome of *Pseudomonas donghuensis* P482, increasing the expression of genes involved in the nitrosative stress response. Additionally, genes related to amino acid catabolism were upregulated. These observations suggest that nitrosative stress may play a role in plant-microbe communication and that the utilization of alternative carbon sources may represent a form of metabolic adaptation.

In this study, we determined the resistance of *P. donghuensis* P482 to nitrosative stress induced by the nitric oxide donors, sodium nitroprusside (SNP), and S-nitrosoglutathione (GSNO), measured as the minimum inhibitory concentration (MIC). We also showed that medium composition affects the sensitivity of P482 to nitrosative stress, as reflected by differences in MIC values. The obtained results provide a basis for further investigation of the mechanisms underlying nitrosative stress resistance in *P. donghuensis* P482.

Additionally, we observed production of the red pigment by P482 upon induction by SNP, what was not known for this strain before. We extracted the pigment using chloroform and performed the LC-MS/MS analysis. The phenotypic analysis of knock-in mutants of P482 have shown the role of 7-hydroxytropolone – an iron scavenger produced by this strain in the pigment production.

Acknowledgment: This research was supported by the statutory funding of the Laboratory of Plant Microbiology Intercollegiate Faculty of Biotechnology UG&MUG, University of Gdańsk.

O.4 – Biochemical characterization of enzymes involved in the synthesis of wax esters and fatty acid ethyl esters in microsomal fractions from Arabidopsis thaliana leaves

Alicja Czyż

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Under the supervision of Katarzyna Jasieniecka-Gazarkiewicz, PhD of the Laboratory of Plant Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

Fatty acid ethyl esters (FAEEs) and wax esters (WEs) are metabolites found in animals, plants, and microorganisms. The main functional role of wax esters in plants is forming a protective hydrophobic layer on the surface to prevent water loss and protect the plant against environmental stressors such as insects or UV light. There is much less knowledge about the roles of fatty acid ethyl esters (FAEEs) in plants, but they were found in pollen, fruit arils, as well as oils and leaves of some plant species. It has been reported that in *Arabidopsis* leaves and wheat roots, enzymes utilizing free fatty acid for synthesis of WEs and FAEEs are present. In wheat roots, this enzymatic activity was strongly stimulated by grass herbicides (alloxydim and haloxyfop), suggesting their role in stress response. However, these enzymes have not been comprehensively characterized yet, which represents the primary focus of this study.

It has been reported that the amount of free fatty acids accumulated in plant membranes increases when plant is subjected to environmental stress. Presence of free fatty acids has been linked to several biophysical changes and to lipid peroxidation that can damage the membranes, therefore excess amounts of free fatty acids can be very harmful for the plants. The enzymes characterized in this work are hypothesized to protect plant membranes by detoxifying excess free fatty acids through their conversion into non-toxic ester forms. The main goal of the presented study was to biochemically characterize the enzymes present in microsomal fractions of *Arabidopsis* leaves that utilize free fatty acid for WEs and FAEEs synthesis. Enzymatic activity assays were performed to evaluate the impact of various parameters, including reaction time, temperature, microsomal protein concentration, pH, and different cations. Furthermore, after establishing the optimal biochemical conditions, the enzymatic profiles were evaluated in plants subjected to diverse abiotic stresses, including high and low temperatures, drought, and salinity. As a result of this study the optimal conditions for the activity of tested enzymes were established. Obtained results demonstrated that the formation of both the WEs and FAEEs was efficiently inhibited by tetrahydrolipstatin (a known inhibitor of lipases), indicating that these enzymes exhibit lipase-like catalytic properties. Finally, the activity of the tested enzymes was strongly stimulated by abiotic stress suggesting their role in plant stress response.

O.5 – The role of phospholipid:diacylglycerol acyltransferase1 (PDAT1) in plant adaptation to high temperature stress

Karolina Słowińska

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Under the supervision of Sylwia Klińska-Bąchor, PhD of the Laboratory of Plant Biochemistry, Intercollegiate Faculty of Biotechnology UG&MUG.

Plants are constantly exposed to various environmental stresses, including high and low temperature, salt stress, drought, and chemical stress. Heat stress negatively affects plant growth and development at different stages of the life cycle. To survive under stress conditions, plants activate a range of physiological and biochemical mechanisms that increase stress tolerance and help maintain productivity. Adaptation to heat stress involves stress perception, signal transduction, regulation of gene expression, and physicochemical changes in the cells.

Phospholipid:diacylglycerol acyltransferase (PDAT) is an enzyme involved in the acyl-CoA-independent pathway of triacylglycerol (TAG) synthesis by transferring acyl groups from phospholipids to diacylglycerol (DAG), producing TAG and lysophospholipids. Therefore, its action contributes not only to storage lipid biosynthesis but also to membrane lipid remodeling, a process that may plays a significant role in plant responses to abiotic stress.

The aim of this study was to expand knowledge of the physiological role of PDAT1 and to investigate the adaptation of Arabidopsis thaliana mutants with different AtPDAT1 expressions (overexpressor and knock-out lines) to high temperature stress conditions of varying duration. The study included the phenotypic observations of aboveground plant parts and root development in tested and control (wild-type, Col-0) lines cultivated under both in vivo and in vitro conditions. Moreover, the lipid profiles of leaves and seeds were analyzed using gas chromatography.

The results showed that PDAT1 OE lines exhibited weaker root development under stress conditions, while under standard conditions this phenotypes was observed only during first two weeks of development. In in vivo conditions, after one week of heat stress, PDAT1 OE lines showed improved growth, whereas pdat1 mutants gradually outperformed PDAT1 OE plants following recovery to standard conditions. Prolonged heat stress led to a progressive reduction in yield. One week of heat stress resulted in reduced levels of palmitic (16:0), linoleic (18:2) linolenic (18:3) acids and increased in long-chain fatty acids (20:1, 22:1), whereas pdat1 lines showed no differences compared to control line. In contrast, five-week heat stress maintained the PDAT1 OE differences in long-chain fatty acids. In addition, the level of oleic acid (18:1) decreased in all lines, while pdat1 seeds showed increased levels of 16:0 and 18:3 fatty acids.

All collected results suggest that the PDAT1 enzyme may play an important role in plant adaptation. The pdat1 lines appears particularly interesting showing improved growth and a higher 18:3 content, a beneficial omega-3 fatty acids, even under prolongated heat stress.

Acknowledgment: This research was partially financed by the National Science Centre, Poland, project OPUS26, number: 2023/51/B/NZ3/00253.

O.6 – The influence of drought stress on the development of Arabidopsis plants with varied expression of acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT) encoding genes

Maksymilian Patoła
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Drought stress is a deficiency of water leading to significant changes in plant growth and development. It affects numerous plant structures and processes, including lipid metabolism and cell membrane composition.

Acyl-CoA:lysophosphatidylcholine acyltransferases (LPCATs) are enzymes transferring fatty acids from acylCoA to lysophosphatidylcholine in the forward reaction or from phosphatidylcholine to CoA in the reverse reaction. They play an important role in the Lands cycle and LPLAT cycle. Arabidopsis thaliana possesses two genes encoding LPCATs.

The main objectives of the study were to assess the influence of drought stress on the development of *Arabidopsis* plants with varied expression of LPCAT encoding genes and to perform lipid analyses of roots, leaves and seeds of those plants grown under drought stress.

Five plant lines were used in the study: *Arabidopsis thaliana* ecotype Columbia-0 (WT), LPCAT1 knockout mutants – *A. thaliana* SALK_017176C (lpcat1 76), *A. thaliana* SALK_123480C (lpcat1 80), LPCAT2 knockout mutants – *A. thaliana* SALK_004728C (lpcat2 28), *A. thaliana* SAIL_1213_G01 (lpcat2 G01). Plants were grown in in vitro cultures on solid media with polyethylene glycol (PEG) and NaCl to evaluate the effect of osmotic stress on root length. Lipids were extracted from roots of plants grown in in vitro cultures in liquid media with PEG and NaCl, as well as leaves and seeds of plants grown in soil under drought stress using the Bligh and Dyer method. Parts of the root extracts were separated into lipid classes using thin layer chromatography. Full and separated extracts, in the form of fatty acid methyl esters, were analysed using gas chromatography.

The results show a greater root length reduction during osmotic stress in lpcat1 80 and lpcat2 28 mutants when compared to wild type. The effects of mutations on lipids of plant subjected to drought stress were varied. The overall fatty acid content was generally lower in roots of in lpcat1 80 and lpcat2 28 mutants but not in leaves or seeds when compared to wild type. The overall fatty acid composition in roots and leaves was similar in wild type and mutants, however in seeds lpcat1 80 and lpcat2 28 mutants exhibited lower levels of 18:2 fatty acid and higher levels of 20:1 fatty acid when compared to control.

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O.7 – Optimization of recombinant West Nile Virus NS2B-NS3 protease production in a bacterial gene expression system

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West Nile Virus (WNV) is currently one of the most geographically widespread mosquito-borne pathogens globally. It belongs to the *Flaviviridae* family and *Orthoflavivirus* genus, along with other major human pathogens such as the dengue, Zika, and Japanese encephalitis viruses. Factors such as climate change, expanding mosquito vector habitats, and global travel contribute to higher flavivirus transmission rates, resulting in outbreaks in new regions, including Europe. While most West Nile virus infections are asymptomatic or cause only mild, flu-like symptoms, the disease can progress to life-threatening neuroinvasive conditions like encephalitis or meningitis. Altogether, the virus remains a serious public health concern, as there are still no antivirals or vaccines approved for human use.

Structurally, the WNV RNA genome encodes a single polyprotein precursor that must be cleaved into individual units to produce functional viral proteins. Responsible for that cleavage is the NS2B-NS3 protease complex, which consists of the NS2B cofactor and the NS3 protease domain. Since this step is essential for replication and the enzyme is highly conserved across the genus, the NS2B-NS3 complex has therefore emerged as a primary target for drug design. However, its recombinant production in *E. coli* is challenged by poor solubility, low yields and autoproteolysis.

The goal of this project was to develop an optimized workflow to produce high-quality and active NS2B-NS3 protease suitable for inhibitor screening. *E. coli* BL21 cells were transformed with a His-tagged WNV fusion construct (Addgene #204794) with a K104A mutation to prevent self-cleavage and verified via restriction analysis. Optimization of protein production involved testing various parameters including different lysis buffers, IPTG concentration, induction temperature, and its duration. Following cell lysis by sonication, expression and solubility were assessed by SDS-PAGE and Western blotting. An overnight induction at 4 °C (OD600 = 1.25) was selected as optimal, providing soluble and mature WNV NS2B-NS3 protease with minimal degradation. Different IPTG concentrations (0.1–1.5 mM) did not significantly impact final yield, and similarly, lysis buffers containing a reducing agent versus a detergent showed no difference in maintaining enzyme solubility.

His-tagged protein was purified via immobilized metal affinity chromatography (IMAC) on nickel resin, with its identity and purity verified by SDS-PAGE, Western blotting, and Coomassie Blue staining. As the next step, a fluorogenic activity assay is planned to validate the activity of the purified protease and confirm that it is both stable and catalytically active.

To summarize, this project provides an optimized NS2B-NS3 protease complex production workflow for future inhibitor screenings and the identification of potential West Nile virus antivirals.

O.8 – Development of a potential mRNA vaccine against infectious Bronchitis Virus in chickens

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Infectious Bronchitis Virus (IBV) is a highly contagious avian *Gammacoronavirus* that primarily affects chickens. It poses a major threat to the poultry industry by reducing egg production and growth performance, while also increasing mortality rates. The aim of this study was to obtain mRNA encoding the S1 gene of IBV. The S1 subunit is a highly variable region of the spike protein located on the viral surface and plays an important role in viral attachment and immune response induction.

To achieve this objective, initially the restriction analysis of two plasmids was performed. One of plasmid served as the donor of the S1 insert, while the second one acted as the vector for in vitro transcription. Following restriction digestion, ligation was carried out to generate a transcription plasmid containing the S1 gene. The next step involved in vitro mRNA transcription. To evaluate mRNA functionality, mammalian cells were transfected using Lipofectamine 'MessengerMAX' reagent. Finally, expression of the S1 protein was confirmed by Western blot analysis.

The obtained results demonstrated that restriction analysis and ligation were successful, resulting in a plasmid of the expected size. In vitro transcription and Western blot analysis also confirmed successful expression of the S1 protein. However, further optimisation of the mRNA construct is still required for the development of a fully functional vaccine and may be the subject of future studies.

O.9 – The FGFR signaling in regulation of DNA damage response

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Estrogen receptor (ER)-positive breast cancer is the most common subtype of this disease in women. Endocrine therapy (anti-ER) based on tamoxifen remains a standard treatment, however, the development of resistance to the applied drug significantly limits its effectiveness. Increasing evidence suggests that, beyond its classical anti-ER activity, tamoxifen may affect genome stability and activate the DNA damage response (DDR). At the same time, FGF (fibroblast growth factor)/FGFR signaling plays an important role in the regulation of cell proliferation, survival, and cellular stress response. A growing body of evidence indicates that FGF/FGFR signaling may play a role in the modulation of DNA damage response.

The aim of this study was to verify the hypothesis that FGFR signaling is involved in the regulation of DNA damage repair mechanisms in ER-positive breast cancer cells and affects the response to endocrine therapy and drugs targeting DNA-damage (PARP inhibitors).

The study was performed using luminal breast cancer cell lines (MCF7) treated with tamoxifen (4-OHT), the PARP inhibitor-talazoparib, and FGF7 as an activator of FGFR signaling. Activation of the DNA damage response was assessed by analyses of expression of DDR-related markers, including: γ-H2A.X, pBRCA1, pChk1, and PAR/PARP by Western blot analysis and immunofluorescence. The interplay between FGFR signalling, tamoxifen and talazoparib was also evaluated in functional studies focused on adhesion-independent cell growth.

The obtained results demonstrated that tamoxifen treatment induced DNA damage, as evidenced by increased γ-H2A.X levels. Activation of FGFR signaling by FGF7 modulated the cellular response to endocrine stress and exerted a protective effect against tamoxifen, talazoparib, and their combination. Furthermore, FGFR signaling influenced the expression of markers associated with DNA repair pathways, suggesting its involvement in the regulation of DDR mechanisms.

These findings indicate that FGFR activity may contribute to therapy resistance through modulation of the cellular response to DNA damage. Targeting the FGF/FGFR pathway could therefore represent a promising therapeutic strategy to improve the effectiveness of endocrine therapy and PARP inhibitor-based treatment in luminal breast cancer.

O.10 – The role of YAP/TAZ in developing resistance to anti-ER therapy in luminal breast cancer cells

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Luminal breast cancer subtype, characterized by estrogen receptor (ER) expression, is the most frequent molecular subtype of breast cancer and is associated with the most favorable prognosis due to the effectiveness of anti-estrogen therapies, such as tamoxifen. However, intrinsic and acquired resistance to anti-estrogen therapy remains a major clinical challenge. The tumor microenvironment (TME), including cancer associated fibroblasts (CAFS) which secrete fibroblast growth factors (FGFs), modulates resistance to tamoxifen in breast cancer. FGFs act as ligands for Fibroblast Growth Factor Receptor (FGFRs). FGFR2 signaling contributes to endocrine resistance in luminal breast cancer. Although it has been shown previously that YAP/TAZ activity can be induced by growth factor signaling independently of canonical Hippo pathway and FGF/FGFR can promote YAP nuclear translocation, the role of YAP/TAZ is not well understood.

This study aimed to investigate the role of YAP/TAZ in mediating resistance to anti-ER therapies in luminal breast cancer.

Experiments were performed on MCF7 cells and T47D cells. For Matrigel® spheroid assay, T47D cells were cultured for 14 days with DMEM and: FGF7, 4-hydroxytamoxifen (4-OHT), combination of FGF7 and 4-OHT, the YAP/TEAD interaction inhibitor IK-930, IK-930 with FGF7, IK-930 with 4-OHT or IK-930 with 4-OHT and FGF7. Western blot analyses were performed on T47D cells and MCF7 cells. To assess YAP and TAZ expression, T47D cells were starved overnight in FBS-free DMEM, then stimulated with FGF7 for 8, 24 and 48 hours. To investigate post-translational modifications of YAP, T47D cells were stimulated with FGF7 for 10, 30 and 60 minutes. To evaluate YAP subcellular localization after FGF7 stimulation, nuclear and cytoplasmic fractions were isolated. Additionally, MCF7 cells were stimulated with FGF7, 4-OHT and mix of FGF7 and 4-OHT to assess FGF7 and tamoxifen influence on active YAP level. In silico analysis were conducted using KM plotter.

Results indicate that inhibition of YAP/TEAD interaction abrogates protective effect caused by stimulation with FGF7 on T47D cells treated with 4-OHT. Further, YAP protein level was elevated after stimulating T47D cells with FGF7 for 24 hours and TAZ protein level was decreased. Additionally, study demonstrated that stimulation of T47D cells with FGF7 for short period of time increased active YAP and decreased inactive forms of YAP. Further study demonstrated that level of active YAP was increased in nuclear fraction after stimulating cells with FGF7. In silico analysis support these findings and suggested a potential YAP and FGFR2 cooperation in anti-ER therapy resistance.

This study provides evidence that FGF7/FGFR2 signaling mediates resistance to anti-estrogen therapy through activation of YAP. Another conclusion is that YAP and TAZ proteins, despite their cooperation in conventional Hippo pathway, might not always work synergistically. Obtained results could contribute to a better understanding of the molecular mechanisms underlying endocrine resistance in breast cancer.

The study may also support the development of combination therapies targeting both FGFR2 and YAP signaling pathways. YAP activation could potentially serve as a predictive biomarker of poor response to endocrine therapy.

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O.11 – The significance of regulatory interactions between the FGFR and HER2 pathways in the progression of luminal breast cancer

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Breast cancer is a highly heterogeneous disease, with Luminal B tumors representing an hormone receptor-positive subtype characterized by high proliferation and a propensity for therapeutic resistance. Phenotypic plasticity serves as a major driver of this clinical challenge, enabling cancer cells to transition into more aggressive states. Specifically, the fibroblast growth factor receptor 4 (FGFR4) axis has been implicated in driving a molecular switch from a Luminal B phenotype toward an aggressive, HER2-enriched-like state via the downregulation of hormone receptors and hyperactivation of downstream MAPK and PI3K signaling cascades. While targeting FGFR4 with selective inhibitors presents a promising therapeutic avenue, the tumor microenvironment frequently provides alternative survival signals that compromise treatment efficacy.

The aim of this study was to investigate the functional role of the FGFR4 axis and evaluate how a diverse panel of microenvironmental growth factors modulates phenotypic plasticity and targeted therapy response in Luminal B breast cancer models.

Using the T47D cell line as a representative Luminal B model, cells were treated with the selective FGFR4 inhibitor Roblitinib in the presence of a diverse panel of relevant microenvironmental ligands, including amphiregulin (AREG), heregulin (HREG), FGF17, FGF19, and EGF. Molecular changes in growth factor receptors protein levels were assessed via Western blot, while functional impacts on anchorage-independent growth and survival were assessed using 3D soft agar and Matrigel colony-formation assays. Western blot analysis revealed that; stimulation with EGF-family ligands—particularly HREG and AREG—downregulated the receptor expression profiles of EGFR, HER2, and HER3, and upregulated FGFR4 protein levels. Functional 3D assays provided evidence that growth of BCa cells in soft agar and Matrigel was restored by, HREG and AREG in Roblitinib conditions driving statistically significant compensatory colony growth despite FGFR4 blockade.

In conclusion, these findings establish a functional foundation for microenvironment-mediated resistance in Luminal B breast cancer. The results demonstrate that external growth factors, most notably through the HREG/HER3 axis, can potentially create a molecular bypass loop to survive targeted monotherapy and lead to BCa progression.

O.12 – The role of FGFR4 - Aurora A – RAD51 interaction in the response of triple-negative breast cancer cells to anti-cancer drugs

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Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype, defined by the absence of estrogen receptor, progesterone receptor and lack of human epidermal growth factor receptor 2 overexpression or amplification. TNBC is characterized by rapid metastasis and poorer prognosis compared to other subtypes.

PARP inhibitors have shown promise for treating patients with TNBC carrying BRCA1/BRCA2 mutations. However, BRCA mutation reversion and other resistance mechanisms limit the efficacy of this drug class by restoring functional homologous recombination or activating alternative DNA repair pathways. Development of resistance presents a significant clinical challenge.

Fibroblast growth factor receptor 4 (FGFR4) is part of the fibroblast growth factors family. Aberrant FGFR4 signaling, caused by genetic mutations or overexpression, is observed across various cancer types and can lead to enhanced proliferation and cell migration, while playing a crucial role in tumor progression, metastasis, and resistance to therapy. Previous studies showed that FGFR4 signaling is critical for the response of TNBC cell lines to PARP inhibitors. TNBC cells with FGFR4 knockdown exhibit resistance to talazoparib and show a significant increase in phosphorylated Aurora A kinase.

The aim of this study was to unravel the mechanism of resistance to PARP inhibitors and evaluate the role of FGFR4- Aurora A- RAD51 interaction in the response of triple-negative breast cancer cells to anti-cancer drugs. FGFR4 inhibition leads to an increased phosphorylation of Aurora A and increased levels of YAP and RAD51, a key protein homologous recombination. Resulting elevated levels of RAD51 potentially reduce PARP inhibitor sensitivity through restoration of proficient homologous recombination in cells with FGFR4 knockdown. Obtained results suggest that the FGFR4 - Aurora A – RAD51 interaction mediates resistance of TNBC to anti-cancer agents.

O.13 – Preparation of a research model for the analysis of the occurrence of the plasmid DNA phase separation phenomenon in bacteria

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Cellular organization is vital for the proper functioning of cells. While traditional models focus primarily on membrane-bound organelles such as the nucleus and mitochondria, it is now well established that cells also utilize membrane-less organelles, often referred to as biomolecular condensates, which assemble via Liquid-Liquid Phase Separation (LLPS). Despite lacking an enclosing membrane, these structures can selectively recruit specific proteins while excluding others, thereby ensuring high reaction specificity within the condensate.

In bacteria, which lack membrane-bound organelles, LLPS serve as an ideal mechanism for the spatial organization of cellular processes. Recently, it was demonstrated that the bacterial helicase loader DciA forms condensates with single-stranded DNA and subsequently recruits the DnaB helicase, thereby facilitating its loading onto the DNA. Given that DNA replication is an energetically demanding process that must be tightly regulated and requires high precision, it is probable that phase separation plays a role in its control by spatially separating it from the rest of the cytoplasm. Furthermore, such condensates may increase the local concentration of essential components, enhancing the efficiency of the replication process.

Based on that, the objective of my project was to prepare the research model to evaluate the role of phase separation in the initiation of plasmid DNA replication. To do that, I purified a fusion protein consisting of the TrfA plasmid replication initiator and the Cyan Fluorescent Protein (CFP) to enable tracking via fluorescence. Subsequent biochemical and biophysical assays were performed to evaluate the interaction of the CFP-TrfA with polyphosphate and DNA containing TrfA-specific iterons. Finally, confocal microscopy was employed to characterize the formation and properties of the resulting condensates.

Interaction analysis revealed that while CFP-TrfA interacts with polyphosphate, it does not form specific DNA complexes characteristic of wild-type TrfA. Microscopy confirmed that CFP-TrfA forms biomolecular condensates in crowded mixtures that preferentially localize within polyphosphate granules. Furthermore, within these structures, CFP-TrfA was found to colocalize with both DNA and with the Lon protease, for which TrfA is a substrate.

These results suggest that during replication initiation, TrfA may form biomolecular condensates that locally increase its concentration, thereby promoting efficient plasmid replication. Moreover, the colocalization of TrfA with both DNA and the Lon protease suggests a potential physiological regulatory mechanism: during stress conditions, when polyphosphate is synthesized, these granules may serve as degradation hubs where the Lon protease targets TrfA, thereby arresting plasmid replication under unfavorable environmental conditions. Although further studies are needed to prove that hypothesis, these preliminary data suggest that LLPS could be involved in plasmid replication initiation in bacteria.

O.14 – Construction of vectors for analysis of the intracellular localization of Clp proteases

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Antimicrobial resistance is one of the greatest public health threats worldwide, highlighting the urgent need for novel antimicrobial therapies. One of potential targets for new antibiotic treatments is proteolysis, an essential process responsible for maintaining protein quality control, stress adaptation and cellular homeostasis. In *Escherichia coli*, protein homeostasis relies on ATP-dependent proteases which degrade damaged, misfolded and regulatory proteins under changing environmental conditions. Clp proteases play a particularly important role in stress response and cellular adaptation. Although their biochemical functions have been extensively studied, less is known about their intracellular localization and how it may change under different physiological conditions.

The aim of this study was to construct five pET-based vectors enabling the analysis of the intracellular localization of selected cytosolic Clp proteases – ClpA, ClpX, ClpP, HslU and HslV – in *E. coli* under stress conditions. To achieve this, gene fusions encoding Clp proteases genes fused via linker to self-labeling HaloTag or SNAP-tag genes were designed and assembled using a molecular cloning method – Gibson Assembly. These tags enable specific fluorescent labeling of proteins upon addition of TMR ligand, through Click Chemistry. This allows visualization of each protease localization in living bacterial cells.

All the resulting plasmids were verified by restriction analysis and DNA sequencing to confirm the correct assembly, insertion of target sequences and preservation of the appropriate reading frame. Expression of all fusion proteins was further confirmed by Western blot analysis, using anti-HaloTag or anti-SNAP-tag antibodies, after their overproduction in the *E. coli* BL21(DE3) system upon IPTG induction, as well as by Coomassie Brilliant Blue staining.

The constructed vectors enabled successful visualization of the intracellular localizations of all five Clp proteases in *E. coli* cells.

These findings may provide a basis for further investigation of the spatial organization of bacterial proteolytic machinery and its role in cellular adaptation to environmental changes. Moreover, this research may contribute to future studies on novel antimicrobial therapies, since bacterial proteases such as Clp, represent promising targets in combating antimicrobial resistance.

O.15 – Extension of the plugin functionality for running molecular dynamics simulations in the PyMOL program

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Molecular dynamics (MD) simulations have become an essential tool in structural biology, enabling detailed analysis of protein motion, folding, and interactions at the atomic level. However, the computational demands of MD simulations, particularly for large biomolecular systems, often exceed the capabilities of standard workstations, limiting accessibility for researchers without dedicated HPC infrastructure.

The Dynamics plugin for PyMOL, developed at the Laboratory of Biomolecular Systems Simulation at the University of Gdansk, provides a graphical interface for running GROMACS-based MD simulations directly from the PyMOL molecular viewer. While the plugin significantly lowers the barrier to performing MD simulations, it currently supports local execution only, restricting its use for computationally intensive systems.

This work presents a design and implementation of an extension to the Dynamics plugin, adding remote job submission capabilities via MoleQueue, a local job queue manager developed within the OpenChemistry project. The existing plugin codebase was thoroughly analyzed to identify integration points. Based on this analysis, an extension architecture was designed in which the plugin communicates with a local MoleQueue daemon through the JSON-RPC 2.0 protocol, which in turn handles SSH connection and job submission to a remote HPC cluster running the OpenPBS scheduling system.

The implementation includes a new MoleQueueClient module responsible for establishing the connection, submitting jobs, monitoring their status, and retrieving results asynchronously. A configuration file was introduced to store connection parameters such as server address, username, and remote working directory. The remote_mode flag was added to the SimulationParameters class, allowing the plugin to switch between local and remote execution while maintaining full backward compatibility with the existing workflow.

The proposed extension is intended to make advanced HPC resources accessible to researchers regardless of their technical background, in line with the original design philosophy of the Dynamics plugin.

O.16 – Structural and functional analysis of a potentially pathogenicubiquitin variant encoded by the human UBC gene

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The ubiquitin-proteasome system (UPS) is essential for maintaining protein homeostasis and regulating numerous cellular processes, including protein degradation, stress response, and intracellular signaling. Dysregulation of ubiquitination pathways has been strongly associated with neurodegenerative disorders and other protein misfolding diseases. This study investigates the structural and functional consequences of a de novo Gly123Arg mutation identified in the human UBC gene of a patient with encephalopathy of unknown etiology. The mutation is located within the second ubiquitin repeat of the polyubiquitin precursor encoded by UBC and results in the substitution of glycine with a positively charged arginine residue.

To evaluate the effects of this variant, a combined computational and experimental approach was applied. Structural analyses included molecular modeling, molecular dynamics simulations, and circular dichroism (CD) spectroscopy. Recombinant wild-type and Gly123Arg ubiquitin variants were overexpressed in *Escherichia coli*, purified using SP-Sepharose chromatography, and analyzed in an in vitro ubiquitination assay containing E1 activating enzyme, E2 conjugating enzyme, CHIP E3 ligase, and the Hsc70 substrate. Ubiquitination efficiency was assessed by Western blot analysis.

Computational analyses demonstrated that the Gly123Arg substitution does not induce major conformational alterations in ubiquitin structure. Molecular dynamics simulations revealed no significant structural destabilization or changes in overall protein organization. Similarly, CD spectroscopy showed comparable secondary structure profiles and thermal stability for both wild-type and mutant ubiquitin, with only a slight difference in melting temperature (Tm). Despite the preserved structural properties, functional assays revealed a striking effect of the mutation. Western blot analysis demonstrated that the Gly123Arg variant completely abolished ubiquitination activity in vitro, indicating a severe impairment of ubiquitin function.

These findings suggest that pathogenic ubiquitin variants may disrupt ubiquitination without causing detectable global structural changes. The results highlight the importance of the functional characterization of ubiquitin variants identified in clinical sequencing studies and support the hypothesis that subtle alterations in the ubiquitin sequence may contribute to proteostasis imbalance and the pathogenesis of neurological disease.

O.17 – The study of Hsp70-substrate complex initiated by Class B J-domain proteins

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Protein aggregation poses a major challenge to cellular proteostasis and is linked to ageing and numerous neurodegenerative disorders such as Alzheimer's and Parkinson's disease. To maintain protein homeostasis, cells rely on an extensive chaperone network that assists protein folding, prevents the accumulation of misfolded species, and promotes the refolding or clearance of protein aggregates. In eukaryotic cells, protein disaggregation is primarily mediated by the Hsp70 chaperone system, an ATPdependent machinery that cooperates with co-chaperones to recognize and remodel aggregated proteins. The activity of Hsp70 is tightly regulated by J-domain proteins (JDPs), which recruit Hsp70 to substrates and stimulate its ATPase cycle. Although both Class A and Class B JDPs cooperate with Hsp70 during aggregate clearance, Class B JDPs have been shown to promote more efficient Hsp70 clustering on protein aggregates and to enhance disaggregation activity. The molecular basis underlying this functional specialization, however, remains incompletely understood.

Unlike the canonical Hsp70 ATPase cycle, in which the JDP dissociates following stimulation of ATP hydrolysis, Sis1, the only JDP class B protein in yeasts, has been proposed to stay in the complex with substrate-bound Hsp70. In this model, the interaction between Sis1 and the EEVD motif stabilizes the complex independently of Hsp70 nucleotide state, leaving the NBD of Hsp70 accessible for the recruitment of additional Hsp70 molecules and promoting the formation of dense Hsp70 clusters on protein aggregates required for their disassembly. This project specifically aimed to investigate how the substrate-binding activity of Hsp70 influences the binding with Sis1. To address this question, recombinant variants of Ssa1 were generated, including wild-type protein and mutants affecting ATP hydrolysis and lacking the EEVD motif. Proteins were overexpressed in the Escherichia coli BL21 Codon+ strain and purified by sequential affinity chromatography, including Ni-NTA and ATP–agarose, to obtain functional protein preparations suitable for downstream biochemical analyses. The activity of the purified Ssa1 variants was subsequently evaluated using a disaggregation assay with luciferase aggregates.

The interaction between Ssa1 variants and Sis1 was analyzed using biolayer interferometry (BLI). Distinct Sis1 association and dissociation profiles were observed among the different Ssa1 variants, indicating that in the absence of client protein, Hsp70 binds Sis1 partially like a substrate. However, if Hsp70 is loaded with the client, the binding curve of Sis1 resembles the binding present in the case of Hsp70 mutants lacking the ATPase activity, hence defective in substrate binding. It confirms that the client protein is present in the Sis1-Ssa1 complex.

Altogether, this work provides further insight into the molecular mechanisms underlying Class B JDPmediated regulation of the Hsp70 disaggregation machinery and supports a model in which EEVDdependent interactions contribute to the stabilization of Hsp70 assemblies on protein aggregates.

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O.18 – Identification of protein variants associated with salivary gland tumors

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Salivary gland tumors are rare neoplasms, with approximately 55,000 cases reported globally in 2022 and 530 (0.25% of new cases) in Poland in the same year. They are characterized by heterogeneity, as the World Health Organization (WHO Classification of Tumours, 5th Edition, 2022) distinguishes 21 types of malignant and 15 types of benign salivary gland pathologies, affecting both major and minor salivary glands.

This study focuses on pleomorphic adenoma (mixed tumor) and adenolymphoma (Warthin tumor), which are the two most common benign parotid salivary gland tumors, characterized by slow growth and low risk of metastasis to other organs, as well as a low risk of recurrence or malignant transformation.

The aim of the research was to identify protein variants associated with mixed tumor and Warthin tumor of the parotid salivary glands, in saliva and tissue samples collected from patients (fragments of tumor tissue and tumor-free tissue, the diagnosis of which was confirmed by histopathological examination) and from healthy individuals (control saliva samples). Mass spectrometry-based proteomics with standard sample preparation protocol (Filter Aided Sample Preparation, FASP, with trypsin digestion and StageTips desalting) and bioinformatic analysis was employed, which enabled the identification and characterization of proteins.

Functional analysis with KEGG pathways enrichment of the common part of salivary and tissue proteomes of patients with mixed tumor and Warthin tumor revealed that identified proteins were mostly involved in the formation of intermediate filaments, immune response and inflammation, with keratins being the most abundant.

Protein variants analysis was performed by combining lists of identified peptides based on database search and de novo peptides and then searching against AliceDB (https://alicedb.ug.edu.pl/). No characteristic protein variants were detected that could be used to identify groups of patients based on tumor type, or to differentiate patients with parotid salivary gland tumors from healthy individuals, suggesting that the applied proteomics-based approach is less suitable for investigating protein variants associated with salivary gland tumors.

O.19 – Optimization of the parameters of the chosen chromatographic step to ensure the optimal purity and quality of the obtained protein

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The rapidly growing market and high demand for therapeutic monoclonal antibodies (mAbs) has increased the need for efficient, easily scalable and cost-effective purification techniques.

Currently Protein A affinity chromatography is the industry golden standard for obtaining highly purified monoclonal antibodies (mAbs). Protein A originating from *Staphylococcus Aureus* exhibits high specificity to Fc region of IgG's, allowing for very effective capture of the antibodies. Besides the positives, traditional protein A-based resins also have their downsides, such as high material costs, which force the need for cleaning and reuse protocols to be introduced and limited flowrates resulting in long purification times.

This study's scope is to optimize already existing monoclonal antibody purification step with the use of current knowledge and new generation of materials. Conducted experiments evaluated four potential protein A-based resins (resins A, B, C and D) as new candidates for affinity chromatography step, as well as an additional alkaline wash aimed to reduce impurities in half-product.

Studies analyzed dynamic binding capacity (DBC10%) of chosen resins, mAb recovery and quality attributes of obtained eluate such as host cell protein (HCP) content, residual DNA (resDNA) content, product aggregation by size exclusion chromatography (SEC), product fragmentation by capillary electrophoresis (CE-SDS) and charge variants of the product by cation exchange chromatography. Results of screening of dynamic binding capacity (maximum amount of mAb that can be bound to a set amount of resin) of four different protein A based resins with pre-purified material containing pure mAbs revealed two potential candidates for further testing- resin A and resin B. These resins dynamic binding capacity was tested again, this time on harvest coming from the bioreactor. This step allowed for simulating normal mAbs production environment and shown real dynamic binding capacity value of chosen resins- with potential nonspecific interactions with impurities taking place. After this step quality of aforementioned parameters was assessed.

Both resins again showed comparable dynamic binding capacity values, lower if compared to the previous step which was expected. Both resins also had most of product quality attributes comparable, with similar mAb recovery, product fragmentation and aggregate levels, however resin B exceeded at removing impurities. Resin B was able to obtain product with 10x fold times less host cell proteins content and 36x fold less residual DNA content. Due to high costs of resin B, ability to remove impurities with steps following affinity chromatography in mAbs purification and possibility of addition of impurity removing washes to protein A chromatography, resin A was selected for further experiments. One of ways to improve removal of host cell proteins in protein A chromatography is addition of alkaline wash of pH ~10. In this study three different buffers were tested- 50mM Sodium Carbonate; 50mM Sodium Carbonate, 1M NaCl and 50mM Sodium Carbonate, 3M NaCl. Addition of alkaline wash improved reduction of host cell proteins 2x fold across all three tested buffers, however resulted in reduced recovery of mAbs by 5-7%. Increasing concentration of NaCl resulted in better mAb recovery. Obtained results show possible improvements to currently existing purification process of mAb X.

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O.20 – Optimization of selected cultivation parameters for a genetically modified CHO cell line in order to achieve the desired product quality after the fermentation stage

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Chinese hamster ovary cell line is a mammalian cell line widely used for therapeutic biologics production, as it is capable of human-like glycosylation and proper protein folding. Thanks to its ability to grow well in serum-free suspension cultures, it is scalable for large-scale manufacturing with a low risk of human pathogen contamination. AMBR15 is an automated micro-bioreactor system that is widely used in cell line development in modern biotechnology. It allows to run up to 48 bioreactors at once, which allows to test wide range of variables. Intensified fed-batch culture is an advanced bioprocessing strategy that achieves significantly higher cell densities and product yields within a standard fed-batch bioreactor by utilizing high-density cell inoculation, concentrated nutrient feeding, and optimized media formulation. By compacting the production timeline and maximizing volumetric productivity, this approach drastically increases manufacturing efficiency.

The aim of this study was to improve media formulation to maintain high viability, viable cell count, and achieve high protein yields in Intensified Fed-Batch (IFB) production culture.

This study was conducted by first preparing different cultivation media and feeds, performing cell revival, and then cell splits. After that, cells were suspended in different culture media, and the AMBR15 system was inoculated. The bioprocess was conducted for 10 days. After 10 days, media containing therapeutic protein were collected, filtered, and sent over to the analytical team for analysis. Protein purification using the JANUS system was also conducted.

Results indicate that a key nutrient was missing since the viability of the cells was gradually decreasing starting from the first day of the process. We could also observe a metabolic shift that occurred in cells based on obtained glutamate levels.

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O.21 – Evaluation of the effect of soil extracts on bacteria causing blackleg and soft rot diseases in potato

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Soft rot and blackleg caused by pectinolytic bacteria of the genera *Pectobacterium* and *Dickeya* are one of the most important bacterial diseases of potato worldwide. These pathogens cause significant yield and storage losses of potato tubers by degrading plant cell walls via pectinolytic enzymes. Due to the limited effectiveness of available control strategies and increasing interest in environmentally friendly disease management, attention has shifted to soil-derived factors that may influence pathogen development.

The aims of this study were to evaluate whether chemical soil extracts derived from potato fields with different levels of blackleg and soft rot incidence (suppressive and non-suppressive soils) show inhibitory activity against *Pectobacterium atrosepticum* and *Dickeya solani* in vitro using antibacterial activity assays, and to investigate the effects of extracts on potato resistance to *D. solani* under hydroponic conditions.

Soil samples were collected from eight agricultural fields where potatoes had been cultivated (S4, S6, S7, S8, NS2, NS5, NS9, NS10). The fields were classified as suppressive (S) or non-suppressive (NS) based on disease incidence observed in the fields and information provided by farmers. Soil physicochemical properties were analyzed, including nutrient content, pH, salinity, organic matter, and soil moisture. Potato samples from fields S7, S8, NS9, and NS10 were analyzed for the presence of pectinolytic bacteria. Isolates were screened on CVP medium, and those showing pectinolytic activity were identified using multiplex PCR targeting *P. atrosepticum, Dickeya* spp., *P. carotovorum*, and *P. wasabiae*. Soil extracts were sterilized either by membrane filtration or autoclaving. Antibacterial activity of extracts against *D. solani* IFB0099 and *P. atrosepticum* IFB5399 was assessed using disc diffusion and 96-well minimum inhibitory concentration (MIC) assays. A separate hydroponic infection experiment was conducted. Potato tubers were sprouted in the dark to obtain uniform seedlings and then transferred to Corning vessels maintained under hydroponic conditions. Once plants had developed roots and shoots, soil extract form field N7 was applied, followed by inoculation with *D. solani* IFB0099. Disease development was monitored over time. Physicochemical analysis showed no significant differences between soil samples. Pectinolytic bacteria were detected only in field S8, and all eleven isolates were identified by multiplex PCR as *Pectobacterium carotovorum* / *P. wasabiae*. In antibacterial assays, no inhibition zones were observed in the disc diffusion test, and no growth inhibition was detected in the MIC assay, therefore, MBC was not determined. Hydroponic infection experiments are still ongoing, although preliminary observations indicate a possible growth-promoting effect of soil extracts.

In conclusion, chemical soil extracts did not show direct antibacterial activity under the tested conditions. However, treated plants showed increased vigor, suggesting possible plant growth-promoting effects. The impact of soil extracts on potato resistance under hydroponic conditions remains under investigation. Additionally, results obtained in collaboration with dr Marta Potrykus using microbiome-based extracts suggest a promising biological effect.

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O.22 – Development, optimization and validation of a GC-MS method for the quantitative analysis of short-chain fatty acids (SCFA) as markers of metabolic activity of the human gut microbiome

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Short-chain fatty acids (SCFAs) are key metabolites produced by the gut microbiota, playing an important role in the maintenance of intestinal homeostasis, modulation of immune responses, and regulation of systemic metabolic processes. Increasing evidence indicates that SCFAs are also involved in bone metabolism and may contribute to the pathogenesis of osteoporosis through their effects on osteoclastogenesis, inflammatory signaling, and immune regulation. Consequently, the development of accurate and reliable analytical methods for SCFA determination is of considerable importance for advancing research on microbiome-host interactions and for the identification of metabolite-based biomarkers associated with human disease.

The present study aimed to develop, optimize, and validate a gas chromatography-mass spectrometry (GC-MS) method for the determination of SCFAs. The analytical workflow

included the development of a procedure for SCFA extraction from biological material, optimization of the derivatization process, and establishment of chromatographic and mass spectrometric conditions for compound detection and quantification. Experimental optimization was performed using a Design of Experiments (DOE) approach. Initially, a Plackett-Burman design was applied to identify the critical parameters affecting analytical performance. Subsequently, significant variables were optimized using a Box-Behnken design in order to establish robust and reproducible analytical conditions.

The developed method enabled efficient chromatographic separation and detection of the analyzed SCFAs. Optimization of experimental conditions resulted in improved analytical response and enhanced repeatability of measurements. The optimized and validated method demonstrated satisfactory specificity, linearity, accuracy, stability, and robustness, confirming its suitability for the determination of SCFAs in complex biological matrices. Furthermore, the applied statistical optimization strategy enabled effective evaluation of factor interactions and contributed to improved overall analytical performance.

The proposed analytical approach may serve as a valuable tool for comprehensive profiling of SCFAs in biological samples, particularly in studies focused on the gut microbiome. Accurate characterization of SCFA profiles may contribute to a better understanding of microbial metabolic activity and its relationship with host physiology. In particular, the developed method may support future investigations into the association between gut microbiota-derived metabolites and bone health, including their potential role in osteoporosis. Ultimately, this work may facilitate the identification of novel biomarkers and contribute to the advancement of microbiome-based diagnostic and therapeutic strategies.

O.23 – Biological properties of *Paenibacillus polymyxa* against *Dickeya spp*. and *Pectobacterium spp*. and genomic analysis of selected strains

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Bacterial phytopathogens from the genera *Dickeya* and *Pectobacterium* are the causative agents of blackleg and soft rot diseases, leading to significant economic losses in potato production worldwide. Due to the limited availability of effective control methods and restrictions on antibiotic use in agriculture, biological control using antagonistic bacteria has emerged as a promising strategy. *Paenibacillus polymyxa* strains are considered promising biological control agents owing to their antagonistic activity against phytopathogenic bacteria.

The aim of this study was to evaluate the biocontrol potential of two newly isolated *P. polymyxa* strains IFB9049 and IFB9050 together with reference strains IOR1813 and type strain ATCC842 against two important potato bacterial pathogens: *Dickeya solani* IFB0099 and *Pectobacterium atrosepticum* IFB5399. Additionally, the study characterized the mechanisms of biological activity by investigating the production of virulence factors and analysing a *P. polymyxa* pangenome constructed from 10 publicly available genomes to identify genes related to extracellular enzymatic activity and siderophore synthesis.

The biocontrol efficacy of the *P. polymyxa* strains was evaluated using potato tuber slice assays based on co-inoculation with the phytopathogens. Extracellular enzymatic activity including cellulase, protease, and pectinase production, as well as siderophore synthesis, was qualitatively determined on selective solid media (CMC, milk, PGA, and CAS agar) based on the formation of activity halos. Genomic analysis for genes associated with virulence factors in core genome of *P. polymyxa* species was performed by Bactopia pipeline. Ten publicly available *P. polymyxa* genomes from the NCBI database were used for pangenome construction.

All tested *P. polymyxa* strains demonstrated antibacterial activity against both *D. solani* and *P. atrosepticum*, resulting in a reduction of rot diameter and potato tissue maceration weight. However, *P. polymyxa* strains also exhibited intrinsic tissue-macerating activity when inoculated alone, at a lower level than the phytopathogens. Enzymatic profiling confirmed that all four strains showed cellulolytic and proteolytic activity in vitro. In contrast, pectinase and siderophore production was not detected under laboratory conditions, despite the genomic analysis confirming the presence of the respective genes associated with these activities of pectinases and siderophores besides proteases and cellulases within the *P. polymyxa* core genome.

The findings indicate that the investigated *P. polymyxa* strains exhibit biocontrol potential against *D. solani* and *P. atrosepticum*. Specifically, *P. polymyxa* limits the severity of phytopathogen induced potato maceration by reducing rotten tissue weight, though it does not decrease the diameter of surface spreading. Notably, *P. polymyxa* is capable of degrading potato tissue independently. While the strains synthesize cellulases and proteases in vitro, they fail to produce pectinases and siderophores despite possession of predicted genes for all four traits in core genome of its species. Ultimately, the potential of *P. polymyxa* to exhibit potent biocontrol efficacy without causing maceration in natural environments presents a promising avenue for future agricultural studies.

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O.24 – Interaction of platinum nanoparticles with idarubicin

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Platinum nanoparticles (PtNPs) are drawing significant interest in nanomedicine because of their stability, large surface area, and potential as drug delivery carriers in cancer treatment. Idarubicin, a synthetic anthracycline widely used against leukemia, shows potent antitumor activity by intercalating DNA and inhibiting topoisomerase II. However, its clinical utility is severely hampered by harsh side effects, such as cardiotoxicity, bone marrow suppression, nausea, and secondary malignancies. This study investigates how idarubicin interacts with platinum nanoparticles of two different sizes (5 nm and 50 nm) and evaluates how these interactions alter the drug's physicochemical and biological behaviour.

Fluorescence spectroscopy revealed a sharp drop in idarubicin fluorescence intensity as PtNP concentrations increased. This quenching points directly to a physical interaction between the drug and the nanoparticles. A parallel decrease in free idarubicin in the solution confirmed that the drug binds to the nanoparticle surface. Thermal analysis showed measurable enthalpy changes during this process, pointing to specific physicochemical interactions. These are likely driven by electrostatic attraction between the positively charged idarubicin molecules and the PtNPs.

At higher nanoparticle concentrations, the components began to aggregate. A red shift in the fluorescence emission spectrum indicated changes in the microenvironment of idarubicin, signalling the formation of drug–nanoparticle complexes or aggregates in solution. These findings show that PtNPs can noticeably alter the structural arrangement and stability of idarubicin.

We evaluated the biological activity of these systems using the Ames mutagenicity test. Higher doses of idarubicin expectedly raised the number of revertant colonies, with 2000 ng/plate marking the highest non-toxic mutagenic dose. Interestingly, both the 5 nm and 50 nm platinum nanoparticles lowered idarubicin's mutagenic activity, suggesting that PtNPs might exert a protective or modulatory effect on the drug's biological impact.

Ultimately, platinum nanoparticles interact directly with idarubicin, reshaping its fluorescence, thermal properties, aggregation tendencies, and mutagenicity. These outcomes highlight the potential of PtNPs as modulators or delivery vehicles in chemotherapy, making further testing in cellular and in vivo models a clear next step.

O.25 – Interactions of titanium nanoparticles with doxorubicin and cisplatin

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Cancer is one of the biggest challenges for public health and modern medicine. In 2022 almost 19 million people were diagnosed with and over 10 million people have died because of cancer. Despite groundbreaking research and innovation, the danger this disease poses is a reality for every single person alive today, especially those living in highly developed countries. According to data collected in 2020, 1 in 4 people will be diagnosed with cancer during their lifetime.

Anticancer drugs, otherwise known as chemotherapeutics, can rely on many diverse mechanisms of action, often specific to a certain type of cancer. Doxorubicin, an anthracycline antibiotic, is used to treat many different forms of cancer. It's mechanism of action involves generating ROS (Reactive Oxygen Species), which damage cellular machinery which leads to cell death, intercalation into DNA and interfering with topoisomerase II function, both of which impede replication. This stops the growth of intensely dividing cells, making doxorubicin well suited for fighting cancer cells. The issue lies in the fact that it works nonspecifically, which causes severe adverse effects like damage to the heart and kidneys and myelotoxicity. Cisplatin is an inorganic compound that exhibits cytostatic properties. It forms crosslinks in DNA and stops replication, which makes its effect similar to that of doxorubicin. It also causes hearing loss, heart damage and kidney damage. In order to ameliorate the negative effects of treatment on the patient's body, chemotherapeutics are modified in order to increase specificity and reduce toxicity to healthy cells. However, another solution that can give similar results exists. The current consensus suggests that metallic nanoparticles can serve as a mean of transport for drugs in the patient's body. Thanks to their structure and size they are able to enter cells on their own and are toxic to cancer cells.

The aim of this project is the analysis of interactions between nanoparticles of titanium dioxide and doxorubicin and cisplatin as well as analysis of their effect on the biological activity of both drugs. The results of DLS (Dynamic Light Scattering) show an increase in the hydrodynamic diameter of titanium dioxide nanoparticles in the presence of both doxorubicin and cisplatin, which suggests that drug-nanoparticles conglomerates are formed.

The results of spectrofluorimetric analysis show increased fluorescence of doxorubicin in the presence of nanoparticles which further reinforces DLS findings. Ames test showed that the mutagenic potential of doxorubicin and cisplatin is lowered in the presence of nanoparticles which titanium dioxide nanoparticles themselves did not show mutagenic activity.

All obtained results indicate that titanium dioxide nanoparticles interact with both drugs and have significant effect on their biological activity. This suggests that they can be used as drug delivery vehicles for anticancer drugs, although further research into the matter is needed.

O.26 – Investigation of the role of auxins in the interaction between *Arabidopsis thaliana* and *Pectobacterium betavasculorum*

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Pectobacterium betavasculorum is a plant pathogen causing soft rot and vascular tissue necrosis in many economically important crops, especially in sugar beet, sunflower, and potatoes. Increasing global temperatures and climate instability may further enhance the spread and virulence of these pathogens, highlighting the need to better understand the molecular mechanisms underlying plant-bacteria interactions. Among *Pectobacterium* species, *P. betavasculorum* can modify plant roots. Auxins, particularly indole-3-acetic acid (IAA), are key regulators of root development and may be synthesized *via* multiple tryptophan-dependent pathways in both plants and bacteria. The role of bacterial auxin biosynthesis in root phenotype modulation has been widely described for plant-beneficial bacteria; however, its involvement in interactions with phytopathogenic necrotrophs, including *P. betavasculorum*, remains poorly understood.

Therefore, the main goal of this study was to investigate whether auxin produced by the pectinolytic necrotrophic pathogen *P. betavasculorum* is responsible for root phenotype modulation and to identify the active IAA biosynthesis pathways during plant-bacteria interaction.

A *ΔtrpAB* mutant impaired in tryptophan-dependent auxin biosynthesis was generated and compared with the wild-type strain in *Arabidopsis thaliana* root phenotyping assays. Then, to identify auxin biosynthesis pathways and to measure the production of IAA by bacteria, comparative HPLC analysis was performed. Additionally, to check the possible transport of auxins into the plant, bacterial outer membrane vesicles (OMV) were isolated.

The *ΔtrpAB* mutant failed to induce the characteristic root phenotype observed for the wild-type strain, suggesting involvement of bacterial auxin production in phenotype modulation. Root phenotyping also showed that bacterial effects on root development depend on auxin influx into plant cells. Auxin quantification and comparative analysis suggested that *P. betavasculorum* utilizes multiple IAA biosynthesis pathways, with the indole-3-acetamide (IAM) pathway representing the predominant route. Furthermore, the mutant produced significantly lower IAA levels than the wild-type strain, indicating partial dependence on tryptophan biosynthesis.

These findings demonstrate that auxin production by the pectinolytic necrotrophic pathogen *P. betavasculorum* contributes to root phenotype modulation induced by *P. betavasculorum* and reveal previously underexplored role of auxin-mediated mechanisms in interactions involving necrotrophic bacterial pathogens and expands our understanding of *Pectobacterium* pathogenicity.

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O.27 – The effect of phage-encoded transcription factors of prophage phi027 on the orientation of the flagellar switch and flagellum biogenesis in *Clostridioides difficile*

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Clostridioides difficile is a Gram-positive, spore-forming anaerobic bacterium and a leading cause of healthcare-associated infections worldwide. The pathogenesis of C. difficile is regulated by numerous elementssuch as adherence factors like flagella, type IV pili and surface-layer proteins (SlpA), as well as spore formation. A key regulatory element controlling major virulence factors is the flagellar switch a phase-variable, invertible DNA segment that coordinates both flagellar biogenesis and the production of major toxins TcdA and TcdB. Furthermore, the virulence of *C. difficile* has been modulated by temperate bacteriophages, such as the phi027 prophage commonly found in hypervirulent ribotype 027 lineages. Loss of the phi027 prophage reduces sporulation, adherence, and overall virulence of the pathogen. However, the precise molecular mechanisms underlying this phage-host interaction remain largely unknown.

The aim of this study was to evaluate the role of putative phage transcription factors phi027_05, phi027_06, and phi027_07, encoded by the phi027 prophage, as regulators influencing the flagellar switch orientation and flagellar biogenesis in *C. difficile*.

The research was carried out by constructing a plasmid complementation using Gibson Assembly method. The phi027_05–07 gene cluster was cloned into the pMTL84151 vector, and the resulting construct was then introduced into a prophage-cured C. difficile strain via conjugation. To determine the impact of the phage proteins on flagellar switch, its orientation (ON/OFF ratio) was quantitatively analyzed using qPCR. The assay compared wild-type, prophage-free, control strain harboring an empty vector and the complemented strain.

The quantitative analysis revealed a statistically significant reduction of the ON orientation in the prophage-free strain compared to the wild-type strain. The introduction of the empty vector did not significantly alter this state. In contrast, the genetic complementation with phi027_05–07 genes in the complemented strain restored the wild-type population phenotype, significantly increasing the ON/OFF ratio compared to the control group. Additionally, atomic force microscopy (AFM) was performed on these strains and provided further evidence that complementation with prophage genes influenced the cellular phenotype.

In conclusion, the study demonstrated that the phi027_05–07prophage proteins are involved in controlling the flagellar switch orientation, biasing it towards the active ON state. These findings suggest a regulatory mechanism where bacteriophage-encoded factors modulate host virulence and motility, which might be crucial for the pathogenic success of hypervirulent *C. difficile* strains. A deeper understanding of this phage-host interaction could enable the development of strategies targeting prophage-encoded regulators to attenuate bacterial virulence, opening new perspectives for therapies against *C. difficile* infection.

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O.28 – Recombinant Bacillus subtilis spores as a weapon against *Porphyromonas gingivalis*

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Porphyromonas gingivalis is an anaerobic, pathogenic human oral pathogen responsible for inducing periodontitis. Periodontitis is a set of inflammatory conditions affecting the tissues surrounding the teeth. It is considered the main cause of tooth loss in adults worldwide. Up to 1 billion people worldwide may suffer from it to some extent.

Arginine deaminase (ArcA) is a protein with enzymatic activity produced by some oral bacteria species. This enzyme catalyzes the hydrolysis of L-arginine to produce L-citrulline and ammonia. It has been proven that ArcA derived from *Streptococcus cristatus* has an inhibitory effect on virulence factors expression of *P. gingivalis*. Furthermore, it was specified that a 15 residue long C-terminal fragment of ArcA protein, named „P4", is responsible for this inhibitory effect. Therefore it might be potentially u sed as an agent against development of *P. gingivalis*.

A way of achieving this goal might be the use of the system called Spore Surface display (SSD). This system enables presentation of a foreign protein encoded by introduced sequence on the surface of the spore by creating a fusion protein with one of the spore coat protein. Bacillus subtilis a model Gram-positive, sporulating bacteria is the best candidate to utilize it in SSD system, because it is nonpathogenic, well known and has a history of successful uses for this purpose.

The aim of this study was to construct recombinant *B. subtilis* spores presenting on their surface the P4 peptide derived from ArcA of S. cristatus and then to verify whether the P4 peptide displayed on the spore surface retains its activity in inhibiting *P. gingivalis* virulence factors.

Methodology which was used to achieve this aim can be divided into three main parts. First being the construction of recombinant *B. subtilis* strains which would have the sequence coding for fusion protein of P4 and one of coat proteins. After successful transformation, from recombinant strains spores were obtained, their coat proteins isolated and analyzed using Western Blot technique in order to confirm the presence of the fusion protein. Finally, influence of the recombinant spores on expression of virulence factors of *P. gingivalis* was analyzed. In order to do this, *P. gingivalis* was incubated with recombinant spores for 4 h after which mRNA was isolated, retrotranscribed and analyzed with qPCR. Based on obtained results, relative virulence genes expression was calculated.

In this study, several variants of recombinant spores were successfully produced and the presence of fusion protein confirmed. Furthermore, after the incubation of *P. gingivalis* with recombinant spores downregulation of checked virulence genes was observed in contrast to nonrecombinant spores. This results gives promising basis for further research in this matter.

O.29 – Mapping of transcription factor Xre binding sites

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Clostridioides difficile is a gram positive spore forming anaerobic bacterium. It is a human pathogen capable of producing toxins. Due to prevalence of antibiotic resistance in this bacterium it is a leading cause of healthcare-associated infections. Transfer of genes encoding toxins or antibiotic resistance can lead to creation of new more dangerous strains. Such transfer can be mediated by bacteriophages, which in case of C.difficile are all temperate. Prophage form of virus can also influence the functioning off the bacterium. Research into C. difficile phage phiCDKH01 discovered XRE protein, which is believed to be responsible for maintaining state of lysogeny. So far this transcription factor was proven to bind to one region where it controls expression of itself and two antirepressors.

Aim of this study was to verify whether transcription factor XRE binds to any other fragments of phiCDKH01 genome and it was divided into three main approaches. First approach was carried out by performing EMSA analysis on DNA fragments of phage genome selected on the basis of similarity to the known binding sequence. Second approach was based on cutting phage's genome into smaller fragments that could be used for creation of fragments library. EMSA analysis was used to determine if any of the fragments could bind to XRE protein. The last approach consisted of performing EMSA assay on whole phage genome cut with restriction enzymes and isolating DNA fragments suspected of binding. Those fragments could be later identified by DNA sequencing.

Three new fragments were identified using fragment library method. However those fragments are of considerable size leading to the suspicion of nonspecific binding. The other two methods confirmed the already known binding site and have not yielded new results. However it proved that those methods are viable for further studies of transcription factors. In conclusion, further studies are required into the character of interactions between newly discovered sequences and XRE protein. It is possible that XRE protein is a main repressor of lytic cycle by mainly controlling expression of two previously mentioned antirepressors.

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O.30 – Characteristics of integrase of bacteriophages infecting *Clostridioides difficile*

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Clostridioides difficile is a Gram-positive, spore-forming, toxin-producing anaerobe that can cause serious gastrointestinal diseases. The main risk factor for higher susceptibility to *C. difficile* infection (CDI) is ongoing antibiotic therapy, which causes a decrease in the biodiversity of intestinal microbiota and makes space for *C. difficile* to proliferate. The current treatment for CDI involves the administration of antibiotics, which simultaneously increases the risk of infection recurrence and the development of antibiotic resistance by *C. difficile*.

One of the considered potential new treatments for CDI is phage therapy. All discovered bacteriophages infecting *C. difficile* are lysogenic, which means they can integrate their genomic material into the genome of their bacterial host and replicate as a prophage alongside the bacterium's DNA.

The process of integration takes place in the presence of the phage enzyme integrase. The integrase is a site-specific recombinase, which participates in recombination between two homologous sites: attP and attB, which are respectively in the phage and bacterial genomes. Most *C. difficile* phages were initially discovered as prophages, and their integration sites could be predicted in silico. However, these predictions still need to be confirmed experimentally.

The aim of my work was to demonstrate the activity of the integrase of bacteriophage phiCDKH01 under in vitro and in vivo conditions. During my research, I constructed one vector with the predicted sequence attB and vectors containing the predicted sequence attP and the gene of integrase. Also, there were prepared constructs with only the sequence attP and only the gene of integrase for controls. The obtained vectors were transformed into competent *Escherichia coli* cells. Selected transformants were used to initiate liquid culture for the in vivo experiment. Arabinose solution was added to half of the culture for integrase expression induction, which was also confirmed by SDS- PAGE. The recombination was detected by PCR using specific primer sets combinations. Acquired PCR products were also verified by sequencing. In the in vitro experiment, the obtained vectors were prepared in two conformations: linear and circular, and added in different combinations to the reaction mixture, with the addition of bacterial protein lysate and purified integrase. The results were also verified by PCR.

In the in vivo experiment, the integrase was overexpressed in E. coli. Results obtained from PCR confirmed that the recombination occurred, which was also confirmed by sequencing. Results acquired from in vitro studies were inconclusive and require further investigation in the future.

O.31 – Social interactions between *Dickeya solani* and *Bacillus subtilis*

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Dickeya solani is a phytopathogen causing blackleg and soft rot diseases. In the Laboratory of Molecular Bacteriology of MUG, it was observed that on solid medium, *D. solani* migrates away from *Bacillus subtilis*. The environmental isolate IFB0102 additionally establishes an inhibition zone against the *B. subtilis* swarm, whereas the laboratory strain IPO2222 lacks this ability. Comparative genomic analysis uncovered only seven single-nucleotide polymorphisms between the two strains, indicating that their contrasting behavior most likely stems from one of those SNPs. Notably, one of these SNPs is located in a gene encoding a LysR-family transcriptional regulator, making it a prime candidate for the observed phenotypic divergence.

The aim of this study was to compare the transcriptional profiles of both strains, with a focus on LysR expression levels, and to construct a fluorescent reporter plasmid that enables in vivo visualization of LysR promoter activity during *D. solani* motility. To obtain transcriptomic data, swarm agar plates with co-cultures of both *D. solani* strains and *B. subtilis* were prepared for RNA-seq. Notably, only co-culture samples were successfully obtained. Among the over a hundred differentially expressed genes identified, the LysR-family regulator exhibited the most pronounced difference. This remarkable difference led us to construct a dual-fluorescent reporter vector designed to visualize LysR promoter activity in different regions of the migration pattern during *D. solani* motility.

A pre-existing dual-fluorescent cassette was repurposed to generate a construct that simultaneously reports on the promoter activity of the LysR-family regulator and a constitutively expressed reference gene, allowing visualization of both targets in vivo.

While transformation of *D. solani* was achieved and the presence of the construct was confirmed in primary transformants, the recombinant bacteria exhibited severe growth defects and could not be maintained in culture.

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O.32 – *Drosera zigzagia* extracts towards *Galleria mellonella* and their antimicrobial activity against *Vibrio spp*.

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The increasing outcomes of Vibriosis infections worldwide and growing problem of antimicrobial resistance has escalated the need to search for alternative antibacterial therapies such as using biologically active compounds derived from natural sources. Carnivorous plants of the genus *Drosera* represent a promising reservoir of secondary metabolites (naphthoquinones, flavonoids and phenolic compounds) with potential pharmaceutical applications. Accordingly, this master's thesis focuses on the biological activity of *Drosera zigzagia* extracts as a source of bioactive compounds exhibiting strong bactericidal activity. The study primarily investigated the antimicrobial, antioxidant and toxicological properties of plant extracts obtained from *D. zigzagia* cultivated under controlled in vitro conditions.

To increase biomass development and metabolite accumulation in *D. zigzagia* in vitro cultures, a range of abiotic elicitors was applied: pH range of the medium (5.5 to 5.7), the presence of activated carbon, plant growth regulators 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP), sodium nitroprusside (SNP) and starvation. The extraction of secondary metabolites was carried out using an ultrasound assisted salting-out method with tetrahydrofuran. The antimicrobial activity of the obtained extracts was subsequently evaluated against two pathogenic marine bacterial strains: *Vibrio vulnificus* and *Vibrio alginolyticus*. Antibacterial efficacy was determined through the assessment of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC), enabling the characterization of both growth inhibition and bactericidal potential. Afterwards, the antioxidant properties of *D. zigzagia* extracts were examined using several complementary biochemical assays: using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay, Ferric ion Reducing Antioxidant Parameter (FRAP) and Cupric Ion Reducing Antioxidant Capacity (CURPAC) methods. This evaluation enabled the comprehensive characterization of free radical scavenging capacity associated with flavonoids and phenolic compounds present in the extracts.

The toxicity of the extracts was assessed in vivo using *Galleria mellonella* larvae as a model organism. Experimental group was injected with D. zigzagia extracts, while control group received PBS buffer. Larvae were incubated at 37°C for four days and monitored at 24-hours interval for alternations in mobility, behavioral changes and melanization intensity as indicators of physiological stress and potential toxicity.

The results obtained identified the optimal in vitro conditions for as a 1⁄2 Murashige and Skoog medium with pH 5.6, supplemented with 0.01 mg/L NAA and BAP and a cultivation period of three months. Moreover, tested plant extracts demonstrated strong antimicrobial activity against both selected strains, as well as antioxidant potential. Furthermore, no major mortality effects were observed in *G. mellonella* model organism indicating a favorable safety profile under the tested conditions. In conclusion, findings confirm the significant antimicrobial activity of *D. zigzagia* extract and highlight their ability to act as a source of biologically active secondary metabolites. The observed low toxicity further supports their potential application as promising candidates for the development of alternative antimicrobial strategies and future pharmaceutical research.

O.33 – The influence of *Solanum dulcamara* exposure to chemical contaminants from the group of perfluoroalkyl compounds in the *in vitro* model

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Perfluoroalkyl substances (PFASs) are a group of chemicals that due to their structure and properties are resistant for degradation in the environment and thus are referred as 'forever chemicals'. Perfluoorooctanoic acid (PFOA) is one of the most frequently detected PFAS in soils and simultaneously it is also detected in the largest quantities as compared to other PFAS. This persistent pollutant is considered toxic and cancerogenic, therefore being a threat not only to humans, but to whole ecosystems. Food consumption from contaminated crops is one of the main sources of PFOA for humans. *Solanum dulcamara* is a plant which belongs to *Solanaceae* family, as well as many agricultural species like potato or tomato.

This study evaluates accumulation and impact of PFOA on *S. dulcamara* growth *in vitro*. The experiment was based on the exposure to varying PFOA concentrations in *in vitro* cultures. The study consisted of LC-MS/MS analysis to determine PFOA accumulation in plant tissues and biochemical tests defining changes in stress markers. The research showed that PFOA accumulates in both shoots and roots of *S. dulcamara* with preferential translocation to aboveground tissues. Despite the significant accumulation, plant showed minimal stress responses during the time of cultivation. Obtained results provide new insights for plant stress response to PFOA but also show phytoremediation potential of *Solanum dulcamara*.

O.34 – Assessment of the synergistic activity of silver ions combined with quinone against clinical isolates of *Pseudomonas aeruginosa* with varied antibiotic resistance profiles in planktonic and biofilm cultures

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Pseudomonas aeruginosa is an example of an opportunistic human pathogen and a common cause of infections in patients suffering from chronic wounds. The species is known for its ability to develop multidrug resistance and produce biofilm, which makes it especially difficult to eradicate. Given the growing prevalence of multidrug resistance in *P. aeruginosa* isolates, it is crucial to develop new methods to combat this pathogen. The project originates from the urgent need for new antibacterial strategies to treat chronic wounds, as well as previous studies demonstrating the synergistic bactericidal activity of quinone compounds - particularly naphthoquinones - in combination with silver against this pathogen.

The aim of this project is to evaluate the susceptibility of clinical isolates of *P. aeruginosa* to synergistic combinations of the simplest quinone structure, 1,4-benzoquinone (BQ), and ionic silver (Ag^+), as well as to determine whether these combinations can eradicate biofilms produced by these strains.

To achieve this goal, the Minimum Inhibitory Concentration (MIC) of both Ag^+ and BQ was first determined for all tested strains, including two reference strains and ten clinical isolates with varying antibiotic resistance profiles. Based on the MIC results, a two-dimensional microdilution assay was then applied to generate concentration gradients of Ag^+ and BQ in planktonic cultures of the tested strains. Bacterial survival following treatment was assessed by monitoring growth curves through OD_{600} measurements for 24 hours. The resulting data was subsequently analyzed using SynergyFinder 3.0 to calculate synergy scores.

To assess synergy in biofilm eradication, a two-dimensional microdilution experiment followed by crystal violet staining is currently being performed on isolates selected based on their biofilm-forming capabilities.

The tested clinical isolates in planktonic cultures exhibited differences in MIC ranges for both BQ and Ag^+, as well as in their susceptibility to BQ-Ag^+ combination. However, no clear correlation was observed between multidrug resistance and lower susceptibility to BQ, Ag^+, or their combinations. Evaluation of the ability of the BQ-Ag^+ combination to eradicate biofilms is still ongoing.

O.35 – Enterotoxin C gene expression in the interaction of *Staphylococcus aureus* with keratinocytes

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Staphylococcus aureus is estimated to colonize 30% of the global population; however, carriage rates increase to 80-100% in a cohort of atopic dermatitis (AD) patients. Treating *S. aureus* infection in barrier-impaired skin poses a significant challenge due to its ability to adopt a facultative intracellular lifestyle, allowing the bacterium to invade and persist within host cells, including keratinocytes. Such mechanism has been proposed as an immune evasive strategy, leading to the development of recurrent and persistent infections. Importantly, enterotoxin producing *S. aureus* strains have been reported with higher prevalence among isolates obtained from AD patients than from healthy individuals. Staphylococcal enterotoxins (SEs) are a class of potent superantigenic exotoxins capable of inducing non-specific T-cell activation, resulting in excessive cytokine release and pro-inflammatory responses. Furthermore, epidemiological studies conducted in Laboratory of Photobiology and Molecular Diagnostics on population of *S. aureus* isolated from AD patients from the Pomeranian region identified staphylococcal enterotoxin C (*sec*) gene as one of the most frequently detected among SE type.The ddominance ofSEC-positive strains potentially points to its significance in host-pathogen interactions. However, while SEC role as a superantigen is well-studied, its putative role in the internalization mechanism remains unexplored.

Therefore, the present study aims to evaluate the role of *sec* gene expression in the internalization of *Staphylococcus aureus* using an *in vitro* infection model of human keratinocytes with filaggrin gene silencing.

HaCaT shFLG keratinocytes, reflecting a key genetic susceptibility factor associated with AD development, were co-cultured with three MW2-derived *S. aureus* strains differing in *sec* expression. SEC production was initially analyzed in a bacterial culture model via SDS-PAGE and Western blot analysis. Following infection and elimination of extracellular bacteria, the intracellular fraction was recovered and quantified to assess bacterial internalization. Relative *sec* expression in recovered intracellular bacteria was evaluated by RT-qPCR, whereas host cell-derived cytoplasmic fractions collected and analyzed for the presence of the target protein by Western blotting.

Western blot analysis confirmed SEC production in the bacterial culture model, validating the applied experimental system. Both MW2 and MW2Δsec strains efficiently infected HaCaT shFLG keratinocytes; additionally, quantitative analysis revealed a statistically significant reduction in intracellular bacterial recovery for the MW2Δ*sec* strain compared with the wild-type control, indicating a potential contribution of SEC to the internalization process. RT-qPCR analysis demonstrated that *sec* expression was activated during keratinocyte infection. Importantly, SEC protein was detected in host cell-derived cytoplasmic fractions obtained from infected keratinocytes, confirming intracellular enterotoxin production during host–pathogen interaction.

Collectively, these findings provide preliminary evidence that SEC may participate in processes beyond its established superantigenic activity and could constitute a factor contributing to *S. aureus* adaptation dduring infection Additionally, the results support the use of the applied research model for future functional studies of keratinocytes response and molecular mechanisms involved in *S. aureus* infection.

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O.36 – The effects of green light irradiation on the A549 human non-small cell lung cancer cell line

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Lung cancer has the highest incidence and mortality rates of all malignant tumors. Over 50% of cases are diagnosed in stage IV, with a median survival rate of 10-12 months. Treatment significantly reduces patients' quality of life. Therefore, alternative treatment options are needed. Photodynamic therapy (PDT) involves the administration of a photosensitizer and its subsequent activation with light of a specific wavelength. This leads to the formation of reactive oxygen species (ROS), which selectively destroy cancer cells with minimal impact on healthy cells. Simultaneously, PDT is also associated with minimal side effects for the patients and a lower risk of drug resistance than commonly used anticancer therapies. Rose bengal (RB) is a well-known photosensitizer from the xanthenes family that can be activated with green light. Its cytotoxic effect has already been confirmed on various cancer cell lines.

The first aim of the study was to assess the effect of 522 nm green light on A549 lung adenocarcinoma cells. The next step was to determine the concentration of RB exhibiting selective photocytotoxicity towards A549 cells while maintaining low toxicity towards normal BEAS-2B bronchial epithelial cells, which enabled the assessment of the compound's selectivity. The results from these experiments were obtained using the MTT viability assays. The final goal of the study was to investigate the effect of RB activated by a selected dose of 522 nm green light on the cell cycle progression and apoptosis induction in A549 cells by flow cytometry analysis.

No significant differences in viability were observed between 6 tested green light irradiation times for both BEAS-2B and A549 cells. For further experiments, an exposure time of 20 minutes was chosen. RB activated with 522 nm green light at concentrations of 10–25 μM showed selective photocytotoxicity to A549 cells, while leaving non-cancerous BEAS-2B cells unharmed. Flow cytometry analysis confirmed that green light-activated RB at 10 mM affects the A549 cell cycle. Furthermore, it significantly increases late apoptosis and reduces the fraction of viable A549 cells, with only minimal effects in BEAS-2B cells.

The obtained results indicate the potential use of green light-activated RB in further research on lung cancer.

O.37 – The effects of red light on neural stem cell proliferation and differentiation *in vitro* under normal and pathological conditions

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Neural stem cells (NSCs) and neural progenitor cells (NPCs) play a crucial role in neurogenesis and proper nervous system development. Disturbances in NSC/NPC function may contribute to the development of abnormalities associated with neurodevelopmental and neurodegenerative disorders, including Autism Spectrum Disorder (ASD). The etiopathology of ASD remains largely idiopathic because of its multifactorial nature involving numerous factors influencing proper brain development.

Photobiomodulation (PBM) is a therapeutic approach showing potential for ASD treatment. As a non-invasive method, PBM uses red or infrared light to stimulate biological processes. PBM has documented anti-inflammatory and antioxidative effects and increases ATP production. Furthermore, PBM inhibits apoptosis and reduces oxidative stress. It also exerts specific effects on neural cells, including enhanced neurogenesis and synaptogenesis, as well as activation of neuroprotective mechanisms.

The aim of this study was to investigate the effects of PBM with 632-nm red light on cultured Neural Stem and Progenitor Cells (NSPCs) derived from wild-type and ASD mouse models.

Primary cell cultures used in this project were isolated from murine embryonic brains collected at embryonic day E12.5. Wild-type cultures (B6) were established from embryos of the C57BL/6 mouse strain, while the ASD model cultures were from embryos of the BTBR T+ Itprtf/J mouse strain. Primary cells were cultured in selective media promoting NSPCs growth and exposed to 632 nm red light at two energy densities, 8 J/cm² and 88 J/cm², for 20 minutes over three consecutive days with 24-hour intervals. Two assays were performed. First, the primary neurosphere formation assay was performed to analyse NSPCs' self-renewal capacity and proliferative potential. Results were assessed by neurosphere count and morphological classification, including size, shape, adherence, differentiation, and stability over time. Secondly, the effects of 632 nm red light on cell differentiation after red light exposure and growth factor withdrawal from the medium were studied by measuring neural outgrowths with membrane and viability staining, followed by fluorescence and microscopy.

Red light exposure maintained NSPCs self-renewal capacity and inhibited differentiation, as irradiated neurospheres remained rounded, compact, and non-adherent. The 88 J/cm² condition showed a stronger effect when compared to the 8 J/cm² exposure. Under differentiation conditions, light exposure promoted neuronal differentiation in B6 cultures, whereas BTBR cultures showed morphological features suggesting enhanced differentiation toward glial cells.

These findings may contribute to the development of therapeutic strategies to reduce ASD-related effects and improve treatment approaches.

O.38 – Investigation of the effect of photodynamic inactivation on the transfer of drug resistance genes via conjugation in *Escherichia coli*

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Antimicrobial resistance (AMR) is one of the top global public health and development threats. The growing prevalence of multidrug resistant strains of *Enterobacterales*, including *Escherichia coli*, is especially alarming. Genes responsible for those mechanisms usually spread through conjugation, since they are frequently located on plasmids. One of the promising methods for combating AMR is antimicrobial Photodynamic Inactivation (aPDI), together with antimicrobial Blue Light (aBL). Therefore, the objective of this study was to evaluate the effect of aPDI and aBL on the transmission of antibiotic resistance genes among *E. coli*.

Sublethal doses of aBL (415 nm) and aPDI (red light 632 nm and Methylene Blue) were established. Consequently, conjugation of two strains of *E. coli* was conducted. The MG1655/pTF2 strain was the donor of the plasmid which carried resistance to cefotaxime, while the J53-2 strain was the recipient strain resistant to rifampicin.

Before conjugation the recipient strain was subjected to sublethal doses of aBL and aPDI. Expression of *tolC* and *rfaC* genes was also assessed after aBL treatment, since the activity of those genes is essential for the proper conjugation process.

Both aBL and aPDI managed to successfully reduce the average frequency of conjugation. Application of red light 632 nm and Methylene Blue alone did not result in any significant changes. Additionally, treatment with sublethal doses of aBL caused major downregulation of *tolC* and *rfaC* genes.

In conclusion, this study suggests that aBL and aPDI are promising methods in terms of reducing the spread of antibiotic resistance among *Escherichia coli* and potentially other Gram-negative microorganisms.

O.39 – Sublethal antimicrobial photodynamic inactivation modulates efflux pump activity in *Escherichia coli*

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Escherichia coli is a widely distributed bacterium that naturally occurs as part of the mammalian intestinal microbiota and is also frequently found in various natural environments. Although many *E. coli* strains remain commensal, pathogenic variants can cause intestinal and extraintestinal infections. Treatment of these infections is increasingly limited by the acquisition of antimicrobial resistance mechanisms. Among these mechanisms, multidrug efflux systems play an important role, particularly the AcrAB-TolC pump, which belongs to the RND family.

One alternative approach is antimicrobial photodynamic inactivation (aPDI). This method is based on the simultaneous action of a photosensitizing compound, light of an appropriate wavelength, and oxygen. As a result, reactive oxygen species (ROS) are generated, leading to oxidative damage to cellular structures. The effect of aPDI on efflux pump activity has not yet been thoroughly investigated; therefore, evaluating this phenomenon may provide important information on bacterial responses to photodynamic stress and on the potential influence of this method on mechanisms associated with antimicrobial resistance.

The aim of this study was to assess the effect of aPDI using red and green light on efflux pump activity in *E. coli* K-12, with particular emphasis on the AcrAB-TolC system. Two bacterial strains were used in the study: wild-type *E. coli* K-12 and a strain with a deletion of the *tolC* gene, which encodes a component of the AcrAB-TolC pump. Lethal and sub-lethal photodynamic treatment conditions were determined for both strains. Three combinations of light and photosensitizers were applied: green light with a maximum wavelength of 522 nm combined with rose bengal, and red light with a maximum wavelength of 632 nm combined with either new methylene blue or TMPyP. To evaluate the effect of photodynamic treatment on efflux-related responses, a fluorescence assay using Nile Red was performed. In parallel, propidium iodide staining was used to assess membrane integrity.

The obtained results indicate that sublethal photodynamic treatment may affect Nile Red accumulation in *E. coli* K-12, suggesting changes in efflux activity after exposure to selected combinations of light and photosensitizers. It was also confirmed that the observed effects were dependent on the AcrAB-TolC system, while their association with nonspecific membrane damage was excluded.

O.40 – The role of CD73 in the regulation of angiogenesis through lipid metabolism

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Breast cancer is the most common cancer among women and remains a major clinical challenge due to its high heterogeneity and complex tumor microenvironment. Processes such as angiogenesis and metabolic reprogramming, particularly alterations in lipid metabolism, play a crucial role in tumor progression. Recent studies have identified CD73, an ecto-5′-nucleotidase involved in extracellular adenosine production, as an important regulator of neoangiogenesis, which involves the formation of new blood vessels from pre-existing vasculature. Moreover, previous research demonstrated that CD73 knockout may significantly alter lipid metabolism and delay the development of breast cancer. Therefore, the aim of this study was to investigate whether CD73 regulates angiogenesis through modulation of lipid metabolism in endothelial cells. Specifically, this study examined how CD73 inhibition and adenosine receptor stimulation affect the angiogenic potential of endothelial cells and their lipid metabolism.

The study was conducted using human and murine endothelial cell lines as well as breast cancer cell lines representing luminal and triple-negative subtypes. Cells were treated with the CD73 inhibitor and selective agonists of adenosine receptors A1, A2A, A2B, and A3. Angiogenic potential was evaluated using the MTT proliferation assay and the Wound Healing Assay (WHA). Lipid metabolism was analyzed by Oil Red O staining to assess lipid droplet accumulation in endothelial cells, while protein levels were evaluated using Western blot.

The obtained results demonstrated that the CD73 inhibition did not significantly affect endothelial cell proliferation or migration. In contrast, activation of the A2B receptor with BAY, modulated endothelial cell migration in a cell line-dependent manner. In the human HMEC-1 cell line, A2B receptor stimulation enhanced migration and promoted the accumulation of neutral lipids, whereas in murine H5V cell line, it reduced migratory capacity. The endothelial cell proliferation was dependent on adenosine receptor stimulation, particularly through activation of the A1 receptor. Furthermore, the breast cancer cell supernatants increased lipid accumulation in endothelial cells, indicating the importance of paracrine interactions between tumor cells and the endothelium. In addition, adenosine signaling influenced the expression levels of proteins involved in lipid metabolism, including SREBF1 and AMPK, further supporting the role of purinergic signaling in the regulation of endothelial metabolic pathways.

These findings suggest that CD73-dependent adenosine signaling, particularly through the A2B receptor, may regulate angiogenesis by modulating lipid metabolism in endothelial cells. Increased lipid accumulation may support endothelial cell migration and metabolic adaptation within the tumor microenvironment. The presented results expand current knowledge on the relationship between purinergic signaling, lipid metabolism, and angiogenesis in breast cancer and may contribute to the development of novel targeted therapeutic strategies.

O.41 – Selection of laboratory assays for reliable assessment of complement-dependent cytotoxicity

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One of the effector mechanisms of monoclonal antibodies used in the treatment of cancer and autoimmune diseases is complement-dependent cytotoxicity (CDC). To evaluate the ability of a given antibody to activate the complement system, it is necessary to measure the efficiency of the CDC reaction. However, available methods differ in their underlying principles, which affects both the interpretation and reliability of the results. Therefore, choosing the appropriate method is essential.

The aim of this study was to compare three approaches for CDC assessment: flow cytometry with propidium iodide staining, the calcein release assay, and the PrestoBlue metabolic assay. The comparison focused on their sensitivity, specificity, susceptibility to false-positive and false-negative results, and their suitability for assessing the rapid nature of the CDC reaction.

The experiments were performed using Ramos and Raji cell lines, both expressing the CD20 antigen. CDC was induced using rituximab, a therapeutic anti-CD20 monoclonal antibody, while human serum served as a source of complement proteins. Measurements were taken at two time points to evaluate the dynamics of the reaction.

The results showed that the calcein release assay is the most reliable method for measuring CDC. It demonstrated good reproducibility and allows for the simultaneous analysis of multiple samples. However, its main limitation is an increase in background signal over time due to nonspecific dye leakage. In contrast, the PrestoBlue assay was less suitable for CDC evaluation, as it measures metabolic activity rather than direct cell death. Because metabolic signals may persist after cell death, this method can underestimate cytotoxicity. Flow cytometry, despite its high sensitivity and wide applicability, also has limitations. It tends to underestimate CDC because severely damaged cells may be excluded from analysis during measurement. Importantly, the results also showed that the physiological state of the cells and experimental conditions have a significant impact on CDC outcomes.

The conducted study provides insights into the limitations and advantages of individual methods used for CDC assessment, which may facilitate optimization and experimental design, ultimately leading to a more reliable evaluation of cytotoxicity.

O.42 – Modulation of the complement system classical pathway activity by variants of C2 protein

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The complement system is a complex hub-like network of enzymatic cascades that serves an immune surveillance function. It is activated upon detection of threats, such as foreign microorganisms, apoptotic cells, or neoplastic cells. Depending on the activating factor, complement activation occurs via the classical, lectin, alternative, or newly described Grazym K-dependent pathways. All pathways lead to the lysis of the target cell. However, overactivation of the complement system may contribute to the development of diseases such as atypical hemolytic uremic syndrome, C3 glomerulopathy, and systemic lupus erythematosus. Due to the importance of the cascade system's role, there is a need for therapeutics that would selectively target only one of the pathways, preferably at the early stage.

C2 protein is one of the proteins involved at the beginning of the complement cascade, and it has the lowest concentration of the classical pathway's proteins, which makes it a promising potential therapeutic target. In the classical pathway, C2 and C4 proteins are cleaved by C1s and their subunits C2a and C4b together form convertase C3. The C2b, second subunit of the C2 protein, is a structural component that takes part in the convertase assembly. In our previous studies, we obtained and described gain of function mutation R205L in the C2b subunit, which enhances the cytotoxic effect, probably by increasing the affinity of the C2 to C4.

The objective of my work is to generate variants of the C2 protein containing the gain-of function mutation R205L, along with S679A, D561A, and H507A, which were predicted as potential loss-of-function mutations. Such variants potentially could have the activity of a competitive inhibitor and could potentially be used as a therapeutic.

ExpiCHO-S cells were transfected with plasmids carrying genes encoding the C2 variants. Products were then purified using affinity chromatography, yielding clear peaks indicating the presence of the products, which were confirmed by electrophoretic separation.

To date, the C2 S679A and C2 S679A R205L were tested using calcein release assay and convertase assay. In the calcein release assay in human serum depleted of protein C2, variant C2 S679A demonstrated significantly lower enzymatic activity compared to the C2 wild-type protein, and C2 S679A R205L demonstrated enzymatic activity at the level of the background. These findings confirmed the loss-of-function character of the S678A mutations. However, we did not detect competitive inhibition in experiments conducted in full normal human serum.

I yet plan to purify using size exclusion chromatography, C2 D561A and R205L D561A proteins, and re-produce H507A. The remaining variants will undergo functional characterization to evaluate their activity, inhibitory potential, and therapeutic relevance.

O.43 – Development of a GASDALIE Fc-Engineered Anti-C4d Monoclonal Antibody to Overcome Tumor Resistance via Enhanced ADCC

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Ever since the approval of rituximab for oncology treatment in 1997, monoclonal antibodies (mAbs) have become an exponentially growing sector of biotechnology. With more than 100 mAbs approved for clinical practice, they represent the fastest growing group of therapeutics in clinical trials. With constant heavy chain mediating effector functions and variable light chain that binds specific epitopes, mAbs have proven to be a highly versatile tool in oncology, immunology, and diagnostics offering unparalleled efficacy and precision.

However, challenges persist, studies show high treatment resistance in tumor cells after repeated exposure to anti-CD20 antibodies due to CD20 shedding and complement exhaustion. Opportunistically, the activation of the classical pathway of the complement leaves a footprint – C4d protein fragment deposition on the cell membrane of these therapy-resistant cells.

This study aims to manufacture and evaluate a novel application of anti-C4d monoclonal antibody designed to target the C4d fragment and eliminate the cancer cells via enhanced antibody dependent cell cytotoxicity (ADCC). This mAb contains GASDALIE (G236A/S239D/A330L/I332E) Fc-region mutations set to amplify the affinity to FcγIIIa (CD16) receptor expressed on Natural Killer (NK) cells.

The antibody was expressed using ExpiCHO® mammalian expression system and purified through protein A affinity chromatography. To examine its specificity and cross-reactivity, enzyme-linked immunosorbent assay (ELISA) was performed, comparing its biding affinity across C4, C4b and C4d proteins. Furthermore, a complement-dependent cytotoxicity (CDC) assay was performed to assess the antibody's properties and compare it to its CDC directed counterpart.

In collaboration with the Department of Immunology of the Mossakowski Medical Research Institute, Polish Academy of Sciences, in vitro ADCC assays are currently being conducted. These experiments utilize donated peripheral blood mononuclear cells (PBMCs) and NK cells isolated via immunomagnetic EasySep™ cell separation kits to ensure a highly purified effector population. The engineered antibody is evaluated using the wild-type Raji lymphoma cell line pre-treated using Rituximab to induce C4d deposition. Effector-mediated cytotoxicity and specific tumor cell lysis are quantified via flow cytometry tracking DRAQ7 dye infiltration into membrane-compromised target cells. In the near future we hope to examine the antibody's effect on the rituximab-resistant cell lines RR and 4RH.

So far, the results show a specific C4d fragment biding as well as no cross-reactivity with the C4 and C4b proteins. No CDC enhancement was observed for the ADCC directed antibody as expected. ADCC experiments using the wild type of Raji cells did not show an enhancement of ADCC compared to Rituximab. We anticipate that the essays performed on the resistant lines will demonstrate a statistically significant, dose-dependent increase in DRAQ7-positive tumor cell populations during ADCC assays compared to wild-type equivalents. Ultimately, when proven successful, this study will demonstrate a novel application of anti-C4d mAbs, offering a promising strategy for complementary or second-line immunotherapy to rescue patients who have failed classical complement-activating antibody regimens.

O.44 – The role of the β-hairpin region of DNAJA2 in preventing Aβ42 aggregation

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Amyloid aggregation is a hallmark of neurodegeneration.

Amyloid fibrils are protein aggregates, which are characterized by their highly ordered structure and low solubility. Aggregation of amyloid β peptide (Aβ42) into amyloid fibrils is a primary cause of Alzheimer's disease. J-domain proteins (JDPs) have been characterised as one of the most effective inhibitors of this process.

Here, we find that class A JDPs inhibit Aβ42 aggregation more efficiently than their structurally similar class B JDP paralogs. Notably, class B lacks the signature zinc finger domain (ZnF) of class A JDPs. We hypothesize that the ZnF or its β-hairpin fragment, are the driving factors for class A's inhibitory activity. By studying Aβ42 aggregation kinetics of JDP variants using Thioflavin T assay, we identified that β-hairpin fragment is essential to protect Aβ42 oligomers from growing into fibrils, thereby suppressing Aβ42 amyloid elongation. Our results demonstrate an essential role of class A JDP's ZnF and its β-hairpin fragment in prevention of pathological aggregation and underscore the functional complexity of the class A JDPs.

O.45 – In vitro analysis of the interaction between the bacterial IscU protein and the Hsp70 system specialized in the iron-sulfur cluster biogenesis

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Iron-sulfur (Fe-S) clusters are one of the most ubiquitous and ancient prosthetic groups in proteins. These cofactors are highly evolutionarily conserved and present in all living organisms. Fe-S proteins participate in essential biological processes, such as electron transport during respiration, ribosome assembly or DNA repair. The unique properties of these clusters are derived from the ability of iron atoms to switch between oxidation states.

Despite their structural simplicity, the Fe-S clusters biogenesis is a highly complex process. The first step involves the cluster assembly on the molecular scaffold protein. After that, the cluster is transferred to recipient proteins which requires interaction of molecular scaffold with Hsp70 chaperone system. The bacterial Hsp70 system consists of two proteins: a J-domain co-chaperone – HscB and a Hsp70 ATPase – HscA.

The bacterial IscU protein acts as a molecular scaffold during the iron-sulfur cluster biogenesis process. It's a small, evolutionarily conserved protein that coordinates the Fe-S cluster through three cysteine residues. During the transfer of the cluster to recipient proteins IscU interacts with the Hsp70 chaperone system through two binding sites: a MVY region that recruits the J-domain protein HscB and a highly specific LPPVK motif, with the PVK amino acids identified as the core residues, dedicated to HscA binding.

The aim of this project was to examine the interactions between the bacterial Hsp70 chaperone system (HscA/HscB) and IscU protein in wild-type (WT) form and a mutant version (P101A V102A K103A), expressed using two expression vectors: pTrc99 and pVP67K. Structure of four purified proteins was verified using circular dichroism (CD) spectroscopy. The *in vitro* analysis focused on evaluating the IscU-HscB interaction via Biolayer Interferometry (BLI) and the stimulation of HscA ATPase activity through a functional assay.

Despite the differences in thermal stability between the wild-type form and mutant version shown by CD analysis, the BLI analysis confirmed that in all four samples IscU protein retained its ability to interact with the HscB cochaperone. However, ATPase activity stimulation measurements demonstrated that introduced mutation within the PVK motif of the IscU protein reduces its ability to stimulate ATPase activity of HscA, highlighting the critical role of this motif in the interaction.

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O.46 – Measurement of DNAJB1 affinity for the N-terminal region of amyloid-β and crystallisation of the DNAJB1-Aβ complex

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Protein homeostasis is maintained in part by J-domain proteins (JDPs), which are essential for preventing protein aggregation under stress conditions and assisting in the folding of unfolded polypeptide chains. JDPs function as co-chaperones for Hsp70 proteins and, together with them, cyclically bind and release substrate proteins. Their role is to deliver protein substrates to Hsp70 and stimulate the ATPase activity of Hsp70 proteins via the J-domain.

JDPs are divided into three classes: A, B, and C. In humans, an example of a class A protein is DNAJA2, while DNAJB1 is an example of a class B protein. Class A proteins are found in all domains of life and in all cellular organelles. The less numerous class B, which is evolutionarily related to class A, has a similar domain organization. Proteins from both classes consist of the following domains: the J-domain connected through a glycine/phenylalanine-rich (G/F) region to two β-barrel domains- CTD I and CTD II- responsible for substrate binding, and a dimerization domain (DD). Unlike class A proteins, class B proteins do not possess a zinc-binding region within the CTD domain. Class C is comprised of all other proteins that contain a J-domain anywhere in their structure.

One of the substrates that JDPs are binding in order to maintain their conformation are amyloid-β (Aβ) peptides. They are cleaved from a larger precursor, APP (Amyloid Precursor Protein), mainly into two isoforms: Aβ40 and Aβ42. The Aβ42 peptide has a tendency to aggregate into fibrils. These structures are the main components of insoluble amyloid plaques found in the brains of patients with Alzheimer's disease, which is a neurodegenerative disease. Its symptoms include dementia, decline in sleep quality, personality changes, and, in later stages, even complete loss of awareness of recent experiences and surroundings.

The aim of this study is to visualize the DNAJB1-Aβ complex by obtaining its 3D structure using X ray crystallography. For this purpose, I overproduced and purified DNAJB1 CTD domain- our preliminary simulations show that this domain binds the N-terminus of Aβ peptide. I then performed fluorescence anisotropy assays to measure its affinity for two chemically synthesized N-terminal fragments of Aβ42: Aβ1-16 and Aβ1-28. I also attempted to crystallise the apo structure of DNAJB1 CTD by performing extensive crystallisation screens followed by an optimization process.

The results of the fluorescence anisotropy experiments allowed me to estimate the dissociation constant for DNAJB1 CTD-Aβ binding, which enabled me to calculate the concentration of peptides required to form the complex. Moreover, the obtain dissociation constant values show that this binding is weak, but biologically meaningful. Because of the nature of JDPs as co-chaperones, interactions within this complex may be intentionally weak so that it can form and dissociate dynamically. Data collected from X-ray diffraction indicate that further optimization of the crystallisation process is needed to obtain the structure of the substrate-binding domain of DNAJB1 with and without Aβ peptide fragments bound to it.

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O.47 – A Unique N-Terminal Domain Enables Hairpin Unwinding by Efa DNA Polymerase

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Bacteriophages are the most abundant biological entities on Earth. While some phages rely entirely on host replication machinery, others encode their own replication enzymes.

Recently we have characterized Efa DNA polymerase encoded by the bacteriophage vB_EfaS-271 infecting *Enterococcus faecalis*. It belongs to the B-family of DNA polymerases and has a canonical right hand core structure composed of the palm, fingers, thumb and exonuclease domains. Additionally, it contains a distinct N-terminal domain which, in case of Efa, is involved in interactions with DNA ahead of the enzymatic core. Based on structural and functional analyses, the N-terminal domain of Efa polymerase resembles a single-stranded DNA-binding (SSB) protein. We have shown that this domain is responsible for Efa's ability to unravel hairpin structures formed within DNA. Accordingly, truncation of the N-terminal domain results in the loss of the polymerase's ability to unwind DNA secondary structures.

The aim of our study was to identify and characterize Efa polymerase variants carrying single and multiple mutations in the N-terminal region in order to determine which amino acid residues are involved in DNA binding. Based on contact points between the N-terminal domain and DNA observed in the solved structure, we selected several amino acid residues that might contribute to DNA interaction and, consequently, to hairpin unwinding. Our objective was to identify a variant that would display activity similar to that of the polymerase lacking the N-terminal domain, while retaining the overall structure characteristic of the wild type.

After analyzing numerous single mutants, we found out that none of them lost the unwinding activity. However, quadruple and octuple mutants exhibited a significant reduction in activity compared with the wild-type protein. These findings suggest that DNA binding and hairpin unwinding by the Efa N-terminal domain are mediated by the cooperative action of multiple residues.

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O.48 – Purification of the ubiquitination machinery components essential for studying the interaction between Salmonella and the host ubiquitin–proteasome system

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Ubiquitination is an essential post-translational protein modification mainly known for signaling proteins for degradation by proteasomes. The process consists of three stages (E1 activation, E2 conjugation and E3 ligation) catalyzed by the corresponding three classes of enzymes and involves large protein complexes with modular components. Its complexity is attributed to its disparate functions within the cell, which includes mediating immune response to bacterial pathogens by regulating the NF-κB pathway.

Pathogenic bacteria are known to target this pathway by use of effector molecules, interfering with and modulating the immune response to enhance their own survival and proliferation.

Salmonella enterica is a major human pathogen and the leading cause of bacterial foodborne illness worldwide. It is known to release over 40 distinct effector molecules into host cells using Type III Secretion Systems. Some of them are known to inhibit the NF-κB pathway at its various stages, including interference with ubiquitination machinery.

To study the effect of these molecules in detail, we aim to reconstruct the Cullin-RING ligase 5 (CRL5) E3 ubiquitin ligase complex *in vitro*. It is a known NF-κB regulator and therefore a potential target for pathogen effectors, making this research significant.

By utilizing methods of molecular cloning, protein overexpression and purification, we aim to obtain multiple components of the complex suitable for *in vitro* molecular assays. This will allow us to closely investigate the interaction with the effector protein and acquire experimental structures of various forms of the CRL5 complex, including with the *Salmonella* effector, elucidating the structural determinants of the interaction.

O.49 – Analysis of changes occurring in tumour-educated platelets (TEPs) as a result of interactions with cells from selected cancer cell lines

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Platelets are increasingly recognized as active contributors to cancer progression. Beyond their role in haemostasis, platelets support tumour development through mechanisms such as proliferation, epithelial-mesenchymal transition, immune evasion and metastasis. Tumour cells can alter platelet RNA composition, leading to the formation of so-called tumour-educated platelets (TEPs). Due to their dynamic transcriptomic profile, TEPs have emerged as a promising source of biomarkers for liquid biopsy-based cancer diagnosis and monitoring. However, despite growing interest in their clinical application, the precise mechanisms underlying platelet education remain insufficiently understood. Moreover, to use TEPs as source of genetic biomarkers, there is also a need to identify and validate unique molecular signatures characteristic for different cancer types.

The aim of this project is to investigate how interactions with different cancer cell lines influence the platelet transcriptome and to determine whether observed transcriptomic changes are specific to particular tumour types or individual cell lines. Additionally, the study aims to evaluate whether exposure to tumour secretome alone is sufficient to induce transcriptomic alterations in platelets and to assess the contribution of platelet activation status to these changes.

To address these questions, an *in vitro* co-culture model was established using selected breast and ovarian cancer cell lines and human blood platelets derived from one healthy donor. Platelets were incubated with cancer cells under different experimental conditions, including direct co-culture, indirect co-culture using a semipermeable membrane system, the presence or absence of foetal bovine serum in the medium and activated versus non-activated platelet states. Following a one-hour incubation, RNA was isolated from both TEPs and cancer cells. After RNA isolation, transcriptomic profiling will be performed using RNA sequencing, followed by bioinformatic analyses to identify differentially expressed transcripts and cancer-associated molecular signatures.

Although final results are not yet available, it is expected that exposure to different cancer cell lines will induce distinct transcriptomic changes in platelets. The study may reveal whether these alterations are cell line-specific or cancer type-specific. Improved understanding of transcriptomic changes in TEPs may contribute to the identification of more robust platelet based biomarkers and support the future development of personalized liquid biopsy approaches in oncology.

O.50 – Optimization of the protocol for detection of circulating tumor cells in breast cancer patients

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Breast cancer is one of the most frequently diagnosed malignancies in women, and one of the main causes of breast cancer-related mortality is the development of distant metastases. Circulating tumour cells, referred to as CTCs, may play an important role in this process. These cells detach from the primary tumour and enter the bloodstream. CTC analysis represents a potential tool for assessing disease progression, metastatic risk, and response to treatment.

The aim of this study was to optimize a protocol for the detection of CTCs in material derived from the peripheral blood of breast cancer patients. Isolated PBMC (Peripheral Blood Mononuclear Cells) fractions, which may contain CTCs, were applied onto adhesive microscope slides, allowing the material to be stored and subsequently subjected to immunocytochemical staining.

In the first stage of the study, the usefulness of immunocytochemical staining based on the detection of a precipitate formed through enzymatic substrate conversion was evaluated. However, the obtained results were unsatisfactory due to high background staining and poorly defined signal. Therefore, the subsequent part of the study was performed using immunofluorescence (IF). An IF staining protocol was established and optimized, enabling the detection of pan-cytokeratins (panCK), vimentin, DAPI, and CD45. In the case of CD45, absence of signal was expected in CTCs, as this marker is characteristic of leukocytes. The developed protocol was then applied to the analysis of control samples obtained from health individuals (n=6) and samples collected from breast cancer patients (n=7).

In samples obtained from two patients, panCK-positive cells were identified; however, these cells also expressed CD45, which makes their unambiguous classification according to the classical criteria for CTC identification difficult. Nevertheless, literature data indicate that, under certain conditions, tumour cells or cells with a CTC-associated phenotype expressing CD45 may occur. This suggests the need for cautious interpretation of the results and further characterization of such cells.

As part of this study, single-cell genome analysis was also performed on cells showing a positive signal in IF staining and collected from the slides, which constituted an initial step toward further molecular characterization of cells suspected of having a CTC phenotype.

The obtained results indicate that immunofluorescence may be a useful technique for the analysis and detection of CTCs on adhesive slides, particularly due to the possibility of collecting cells for further analysis. However, further molecular characterization based on the analysis of mutations and aberrations in the collected cells is necessary, as it may allow for a more precise assessment of the presence of oncogenic alterations.

O.51 – Validation of a nanopore sequencing-based method for genotyping hereditary alpha-tryptasemia (HαT) in patients with Hymenoptera venom anaphylaxis

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Hereditary alpha-tryptasemia (HαT) is an autosomal dominant trait defined by an increased copy number of TPSAB1 encoding α-tryptase, present in approximately 4–6% of the general population. HαT is associated with elevated basal serum tryptase and, of particular relevance for clinical allergology, with increased incidence and severity of Hymenoptera venom anaphylaxis (HVA) and with elevated risk of systemic anaphylaxis and hypotensive episodes in this patient group. Accurate genotyping of the tryptase locus is therefore clinically relevant for risk stratification in HVA patients.

The tryptase locus on chromosome 16p13.3 contains four paralogous genes with ≥97% sequence identity between α- and β-encoding sequences and copy-number variability, which limits the choice of clinically usable genotyping methods. Droplet digital PCR (ddPCR) is the current reference assay but requires instrumentation not broadly available outside specialized centers. Targeted nanopore sequencing offers an alternative by combining long-read resolution of paralogous loci with the accessibility of Oxford Nanopore Technologies (ONT) platforms.

The aim of this study was to validate the nanopore sequencing-based method for tryptase locus genotyping developed by Vaxican Sp. z o.o. by applying it to a clinical cohort of HVA patients (n=111) and comparing the obtained genotypes with the ddPCR reference assay.

DNA from peripheral blood was genotyped in parallel by multiplex ddPCR with BamHI digestion and by the ONT-based assay provided by Vaxican Sp. z o.o., including the targeted primer panel, GridION sequencing protocol and bioinformatics pipeline. Concordance was assessed by Bland–Altman analysis of CN(α) and CN(β); diagnostic performance of the ONT assay for HαT detection (CN(α) ≥ 3) was characterized by sensitivity, specificity and predictive values against ddPCR.

Our initial results defined α/β composition demonstrated that the ONT-based assay correctly discriminates α- and β-tryptase paralogs and yields copy-number estimates concordant with the expected composition, supporting application of the method to the clinical cohort.

It seems that the ONT-based method has potential for HαT genotyping in clinical settings; the ongoing validation in the HVA cohort will determine whether it can serve as an accessible alternative to ddPCR.

O.52 – Identification of amino acids of the Human cytomegalovirus IE1 protein responsible for chromosome binding

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The human cytomegalovirus (HCMV) is a double-stranded DNA virus belonging to the Herpesviridae family. HCMV can be lethal to immunocompromised individuals and may lead to neurodevelopmental disorders in neonates. The virus establishes latency and is a was suggested to have oncomodulatory properties in glioblastoma. HCMV Immediate-Early1 protein is one of the first proteins expressed upon infection and is involved in immune system evasion and transcriptional regulation during the lytic cycle. IE1 has been suggested to participate in tethering of the viral genome and is known to localise on chromosomes in a pattern known as painting – evenly covering chromosomes. Our team found that IE1 in glioblastoma cells can also localise in a chromosome associated spots (CAS) pattern, in which it localises in a form of double spots on chromosomes. The core domain of the protein is responsible for this localisation pattern, but the exact amino acids involved are unknown. We hypothesise that the IE1-chromosome binding is indirect, through an interaction with a cellular protein.

The aim of the study was more precise mapping of IE1 residues responsible for its localisation on chromosomes in the chromosome-associated spots pattern. We mutated selected amino acid residues that could be responsible for protein binding and analysed their localisation searching for loss of CAS pattern.

In cooperation with Professor Adam Sieradzan from the Faculty of Chemistry of UG, we designed two IE1 core domain mutants of residues that were probable binding sites for proteins, with the aim of preventing the binding of the partners. After designing the changes, we cloned the genes encoding the IE1 mutants into vectors with myc and EGFP tags. This resulted in mutant proteins with two different tags, one small tag not affecting the structure of the protein and another allowing for easy monitoring of localisation using fluorescence. For subsequent analyses, we transfected T98G glioblastoma cells with vectors encoding the mutant proteins. We analysed the localisation of mutant proteins on metaphase chromosomes and quantified the observed patterns. We controlled for efficient expression of the mutant proteins using western blot and checked that the mutants localise in the nucleus using immunofluorescence staining.

We noted that one of the mutants does not localise on chromosomes in the CAS pattern, while maintaining stable expression levels. This suggests that the amino acid residues we mutated in it are responsible for the CAS localisation pattern and that these mutations do not disrupt the overall stability of the protein. They also do not affect the nuclear localisation of IE1. This mutant will allow further studies on the importance of the CAS pattern in HCMV infection and latency.

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O.53 – Investigation of immunomodulatory properties of SARS-CoV-2 ORF3d miniprotein

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an etiological agent of the COVID 19 pandemic. It is a single-stranded positive-sense RNA enveloped virus that belongs to the *Coronaviridae* family and *Betacoronavirus* genus. Its genome encodes nonstructural, structural and accessory proteins that are involved in mediation of antiviral host responses. One of those proteins is ORF3d which is a 57 aa protein with two isoforms: ORF3d and the shorter ORF3d-2. Its predicted molecular mass of ORF3d is around 6 kDa and its function is to this day unknown. There is still lacking information about the most crucial aspects of ORF3d and other accessory proteins which is important to uncover to fully understand SARS-CoV-2 mechanism of action. The aim of this study is to evaluate the effects of ORF3d miniprotein on cellular processes, focusing on antigen presentation pathways as well as assessing its intracellular localization.

Transfection of GP2-293 retrovirus-packaging cells was performed in order to obtain pseudotyped vectors that were later used for MJS and CAL51 cells transduction, resulting in cell lines with stable expression of the ORF3d. The vector allowed for co-expression of ORF3d and NGFR (*Nerve Growth Factor Receptor*) gene from a single mRNA as seperate translation products, resulting in NGFR receptor functioning as a cell surface marker of ORF3d expression. After transduction, ORF3d presence was verified via Western blotting. Flow cytometry was used to assess MHC class I and II (*Major Histocompatibility Complex*) levels as well as other surface proteins levels to analyze the effect on protein traffic to cell surface.

Retroviral vectors were successfully produced and used for the gene introduction into MJS and CAL51 cells. NGFR could be detected on the cells surface indicating expression of ORF3d, which allowed positive cell sorting, resulting in stable cell lines. Western blot analysis confirmed presence of ORF3d protein in transduced cells. Flow cytometry assay showed decreased level of MHC I on MJS cells surface while MHC II level remained unchanged in both cell lines.

In summary, the results of this study demonstrate that ORF3d protein disrupts the antigen presentation pathway what manifests in decreased levels of MHC I. Given its role in immune evasion, ORF3d can represent a potential therapeutic target. Collectively, these findings further insight into the molecular mechanisms underlying SARS-CoV-2 pathogenesis.

O.54 – Characterization of recombinant SARS-CoV-2 spike proteins expressed in *Leishmania tarentolae*

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The continuous emergence of new SARS-CoV-2 variants highlights the need for vaccine strategies capable of inducing broader and more durable immune responses. Most currently available vaccines are based on the highly immunogenic but variable S1 subunit of the Spike protein, particularly the receptor binding domain (RBD). In contrast, the S2 subunit contains more conserved regions involved in membrane fusion, including heptad repeat 1(HR1), heptad repeat 2 (HR2), and stem helix (SH), which are considered promising targets for the development of universal coronavirus vaccines.

The aim of this study was to produce and characterize two recombinant SARS-CoV-2 S2-based antigens, HR1-HR2 and HR1-SH-HR2, using the *Leishmania tarentolae* expression system. The constructs were cloned into an appropriate expression vector for protein production in the *Leishmania tarentolae* system and subsequently introduced into host cells via electroporation. Stable transfectants were selected using bleomycin and protein expression was induced with tetracycline for 72 h.

Expression analysis confirmed successful recombinant protein production in the *Leishmania tarentolae* expression system. Consistent with the expected, the proteins were efficiently released into the culture medium, thereby simplifying downstream purification procedures.

The highest protein yield was observed after 72 h of induction. Recombinant antigens containing a C terminal Strep-tag were purified using affinity chromatography and analyzed by western blotting, confirming successful expression and purification of both constructs.

To evaluate the immunogenic potential of the obtained antigens, mice were immunized with three doses of protein. Sera collected from immunized mice at different time points were analyzed using indirect ELISA. Plates coated with recombinant proteins were incubated with mouse sera collected before immunization and after subsequent primary and booster doses. Increased antibody binding was observed in sera collected after immunization, indicating the induction of antigen-specific humoral immune responses.

The obtained results demonstrate that the LEXSY system enables efficient production of SARS-CoV 2 S2-derived recombinant proteins and supports further investigation of conserved S2 regions as potential components of broadly protective SARS-CoV-2 vaccine.