symbioza

Intercollegiate
Biotechnology
Symposium

The 9th Prof. Krzysztof W. Szewczyk

Intercollegiate Biotechnology Symposium

SYMBIOZA

BOOK of ABSTRACTS

The 21st–23rd of May 2021, online

The 9th Krzysztof W. Szewczyk
Intercollegiate Biotechnology Symposium 'Symbioza'
Book of Abstracts
The 21st–23rd of May 2021, online.
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Contents

Warsaw, the 21st of May 2021.

Dear Participants,

I am delighted to welcome you to the first IBS 'Symbioza' held fully online! But first of all, I wish to express my profound gratitude to all the Organizing Committee members, who keep putting all their hearts into creating 'Symbioza' annually. Thanks to them, the previous, 8th edition of the Symposium has been awarded with 'The Conference of the Year 2019' title in the StRuNa competition organised under the auspices of the Ministry of Science. Undoubtedly, there is no 'Symbioza' without people and their commitment.

At the time of the award ceremony, no one could expect that instead of further undisturbed blossoming, our Society would soon get challenged in such a ruthless way by the COVID-19 pandemic. Nevertheless, our team took up the challenge of transferring the whole event to the digital reality and adjust the Symposium to the new circumstances, in a way the entirety of organisms has been adapting to the unstable environmental conditions over past eras. Even though it took us more than one year and lots of effort to plant the seed of the new Symposium appearance, here comes the time for it to burgeon as does a fern sprout during first days of May. Such a number nine-shaped fern sprout has become a mascot of the May-bloomming 9th edition of the Intercollegiate Biotechnology Symposium 'Symbioza'.

Although talking face to face, exchanging handshakes, or enjoying dinners together has been temporarily indisposed to us, there are so many activities we still can fancy as if we were next to each other – we can do Science! We invited seven inspiring keynote speakers, whose lectures you will have an opportunity to listen to during these three days, and there will be a wide range of participants' interesting presentations divided into eight oral sessions. You will also have a chance to admire two unique poster sessions – each poster will be placed in a separate online room, with a possibility of zooming it in and out, or talk to an author via a dedicated video call – we hope it will feel like a face to face meeting! Don't hestitate to use other side activities we prepared for you, such as workshops about publishing scientific papers, an industry lecture of our golden sponsor, Precoptic, or a bioprint exhibition! To make up for this online form, we invite you to our surprise social event, which might be a pleasant makeshift of normality we all miss.

I deeply hope that despite the remote form of the Symposium you will enjoy the upcoming edition, gather new knowledge and get inspired. The ultimate way to reach complacency and contribute to science is being above the obstacles. Stephen Hawking's words of completely different intention, as it may seem, would get so up to date: *Although I cannot move and I have to speak through a computer, in my mind I am free.* I wish us all such strength, passion, and the experience of freedom! I greatly hope all of you reading will always keep developing and burgeoning like the fern sprout.

— **Klaudia Staśkiewicz**

President of the Warsaw Society of Biotechnology 'Symbioza'

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

— Krzysztof W. Szewczyk

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

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

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Organizer

Warsaw Society of Biotechnology 'Symbioza' (WSB 'Symbioza') was established in 2013, thanks to cooperation between students from three major Warsaw universities: University of Warsaw, Warsaw University of Life Sciences and Warsaw University of Technology. WSB 'Symbioza' brings together young life sciences enthusiasts with a particular interest in biotechnology who want to share knowledge and experience with students and scientists coming from around the world. The main purpose of the Society is popularization and increase of consciousness in society about biotechnology by organizing a wide range of activities.

The Intercollegiate Biotechnology Symposium 'Symbioza' (IBS 'Symbioza'), being the Society's oldest and most important event, is a scientific conference addressed to international students and PhD candidates who want to broaden their knowledge on biosciences. For many, this is the first opportunity in their research careers to present their findings to wider audience. The symposium serves also as a forum to network with young scientists around the world, which leads to successful collaborations, internships, etc. The 8^{th} edition of IBS 'Symbioza' was awarded in the StRuNa Competition as 'Conference of the year 2019'.

Feel the Flow is a project which aims to popularize science among the public in an informal and friendly atmosphere, as well as discuss issues that people face pursuing scientific career. This is realized with a series of meetings (recently online) with renowned science communicators from different branches of life sciences - from physics and chemistry through biology to medicine.
BARWy (Biotechnological Aspects of Various Choices) is an event aimed to show high school graduates and freshmen biotechnology students a variety of professional perspectives and possibilities awaiting them in their future careers, be it in industry or academia. During the event, there is a remarkable opportunity to meet representatives of different biotechnology related professions and listen to their successful stories.

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

Symbioza Umysłów (*Symbiosis of Minds*) is the latest project of the Society that is addressed to secondary schools students. During the recent COVID-19 pandemic, when most education was held online and remotely, we organized an interdisciplinary competition aiming to popularise science and promote cooperation and synergic thinking across the borders among people and scientific disciplines.

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Co-organizers

The organizers of the 9th IBS 'Symbioza' have been supported by three biotechnology student organizations affiliated at the three most important Warsaw universities.

Biotechnology Student Interest Group "Herbion" was established in 2003 at the Faculty of Chemistry of Warsaw University of Technology. "Herbion" conveys a number of scientific projects, such as the construction of a bioreactor, biotransformations using enzymes, the use of microalgae in the cosmetics industry, and genetically engineered phage of lactic acid bacteria. Our members also participate in the IGLUNA contest organized by Space Innovation, where they try to create a self-sufficient module to grow edible plants for astronauts. We also popularize biotechnology through science shows and mass events such as the *Science Picnic* of Polish Radio and the Copernicus Science Centre, the SIGs and Student Organizations' Fair, open days at Warsaw University of Technology and Academic Village during yearly student carnival (Juwenalia). Other activities include educational trips, and organizing monthly open online lectures, known as *Meetoza – dzielimy się wiedzą*.

KNBiotech Science Club is a student research organization at the Faculty of Horticulture and Biotechnology at the Warsaw University of Life Sciences (SGGW). Operating since 1997, the club unites students of biological faculties interested in the broadly understood biological sciences, in particular focusing on the field of biotechnology. Members are divided into 5 sections: animal, plant, bioinformatics, enzymatic and chemical, within which they carry out specific projects aimed at developing their interests. In addition to scientific activity, participants of KNBiotech are involved in popularization events, such as the Science Picnic of Polish Radio and the Copernicus Science Center, Days of Warsaw University of Life Sciences and numerous scientific conferences.

Molecular Biology Student Club at the University of Warsaw is one of the oldest and the most well-known student clubs at the Faculty of Biology. Basic activity involves not only weekly seminars regarding a wide range of topics related to molecular biology, but also organizing thematic events like *VirusWeek2020*, *MatureUp* or *ParaSite*. Members of the Molecular Biology Student Club also participate in events popularizing science, such as the *Science Picnic* or the *Night of Biologists*.

Program overview

		track 1*	track 2*
Day 1: Friday	15.00–15.15	Opening	
	15.15–16.10	**PL-1**: Reini F. Luco	
	16.30–16.50	**I-1**: Precoptic Co.	
	17.00–18.00	Oral session (**O-1**–**O-4**) *Cancer research*	Oral session (**O-5**–**O-7**)
	18.05–19.00	**PL-2**: Jan Guzowski	
	19.30–	Social event	
Day 2: Saturday	10.00–10.55	**PL-3**: Łukasz Drewniak	
	11.05–12.05	Oral session (**O-8**–**O-11**) *Genetics*	Oral session (**O-12**–**O-15**) *Medical biotechnology*
	12.15–13.10	**PL-4**: Andrew Lang	
	13.15–14.15	Lunch break	
	14.15–15.15	Poster session 1 (**P-1** – **P-38**)	
	15.20–16.15	**PL-5**: Felix Müller-Planitz	
	16.25–17.25	Oral session (**O-16**–**O-18**) *Microbiology*	Oral session (**O-19**–**O-21**) *Plant biotechnology*
	18.00–18.45	**W-1**: Jan Cools	
Day 3: Sunday	10.00–11.00	Oral session (**O-22**–**O-25**) *Environmental studies*	Oral session (**O-26**–**O-28**) *Molecular biology*
	11.10–12.05	**PL-6**: Anna Bajer	
	12.15–13.15	Poster session 2 (**P-39**–**P-76**)	
	13.15–15.00	Lunch break	
	15.00–15.55	**PL-7**: Philip Tagari	
	16.00–16.30	Prizes & Closing	

* All events happen on or are linked from the Whova platform. Oral presentation sessions are divided into two parallel tracks.

PL – plenary lecture **I** – industry lecture **W** – workshop **O** – oral presentation **P** – poster

Agenda

Friday, the 21st of May 2021.

15:00–15:15 **Opening**

15:15–16:10 **Plenary lecture**

PL-1 More than just a crosstalk, histone marks can be drivers of the changes in splicing observed during the epithelial-to-mesenchymal transition

Reini Fernandez de Luco, *Institute of Human Genetics CNRS, Montpellier (FR)*

16:10–16:30 **Break**

16:30–16:50 **Industry lecture**

I-1 Expanding confocal imaging into the lower magnifications

Peter Drent, *Confocal.nl, Amsterdam (NL) / Precoptic Co., Warsaw (PL)*

16:50–17:00 **Break**

17:00–18:00 **Oral session: Worst kind of immortality – Cancer research**

O-1 The investigation of the T regulatory cells phenotype in chronic lymphocytic leukemia

Marta Śledź, *Warsaw University of Life Sciences (PL)*

O-2 Acquisition of complement-dependent cytotoxicity by type II anti-CD20 therapeutic antibody obinutuzumab

Alicja Kuźniewska, *University of Gdańsk/Medical University of Gdańsk (PL)*

O-3 Autophagy inhibition overcomes cisplatin resistance in human lung cancer cells

Aleksandra Olszewska, *Military Institute of Medicine, Warsaw (PL)*

O-4 The impact of STING activation on neutrophils (Ly6G+ cells) infiltration in two different cancer models

Alina Drzyzga, *Cancer Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice (PL)*

17:00–18:00 **Oral session: On the other side of the lense**

O-5 Microscopic evaluation of the brushed surface of dental ceramics

Katarzyna Kaczmarek, *University of Łódź (PL)*

O-6 Influence of food components on the gastrointestinal digestion of metallic nanoparticles

Maria Hayder, *Warsaw University of Technology (PL)*

O-7 Rotating magnetic field exposure increases antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* strains

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

18:05–19:00 **Plenary lecture**

PL-2 3D cell-culture scaffolds generated using microfluidics

Jan Guzowski, *Institute of Physical Chemistry Polish Academy of Sciences, Warsaw (PL)*

from 19:30 **Social event** (via WonderMe)

Saturday, the 22nd of May 2021.

10:00–10:55 **Plenary lecture**

PL-3 The use of lignocellulosic waste materials for treatment of heavy metal containing wastewaters – discussions on effectiveness and profitability

Łukasz Drewniak, *University of Warsaw (PL)*

10:55–11:05 **Break**

11:05–12:05 **Oral session: It's not my fault, it's Genetics**

O-8 In pursue of a crosstalk between vitamin D receptor and fibroblast growth factor receptors in cells of different origin

Agnieszka Jakuszak, *University of Wrocław (PL)*

O-9 Nrf2 role in immune surveillance

Sara Mikac, *University of Gdańsk (PL)*

O-10 Role of microRNA in the regulation of estrogen receptors expression in adipose tissue of obese and normal body weight individuals

Krzysztof Koźniewski, *Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw (PL)*

O-11 Evolution of double β-barrel J-domain proteins – major co-chaperones of the Hsp70 systems

Milena Stolarska, *University of Gdańsk/Medical University of Gdańsk (PL)*

11:05–12:05 **Oral session: Wait, there's more than COVID! – Medical biotech**

O-12 Hairy citrullinated *Porphyromonas gingivalis* enhances Toll-like 2 receptor activation

Aleksandra Wielento, *Jagiellonian University, Kraków (PL)*

O-13 Oral pathogens synergize with the inflammatory tissue environment to promote gingival fibroblast activation in periodontitis

Elwira Nieboga, *Jagiellonian University, Kraków (PL)*

O-14 Bacterial cellulose as a carrier for oral drug delivery system

Krzysztof Ochał, *West Pomeranian University of Technology, Szczecin (PL)*

O-15 Can diabetes medication help in treating periodontitis patients? Inhibitory effect of metformin on osteoclast formation and activity *in vitro*

Katarzyna Łagosz-Ćwik, *Jagiellonian University, Kraków (PL)*

12:05–12:15 **Break**

12:15–13:10 **Plenary lecture**

PL-4 Gene transfer agents: bacteria tame phage and put them to work

Andrew Lang, *Memorial University of Newfoundland, St. John's (CA)*

13:15–14:15 **Lunch break**

14:15–15:15 **Poster session 1 (P-1–P-38)**

15:20–16:15 **Plenary lecture**

PL-5 The biogenesis and function of the nucleosome landscape

Felix Mueller-Planitz, *Dresden University of Technology (DE)*

16:15–16:25 **Break**

16:25–17:25 **Oral session: Small is beautiful (but small) – Microbiology**

O-16 The structural-functional analysis of complexes of plasmid replication initiation protein, Rep, and the ssDNA

Monika Ciuksza, *University of Gdańsk/Medical University of Gdańsk (PL)*

O-17 Assessment of the toxicological effects of copper and titanium dioxide-based nanomaterials on microbial cells

Oliwia Metryka, *University of Silesia, Katowice (PL)*

O-18 Multiple *parS* centromere-like sites in *repABC* replicons: plasmid pAMI4 of *Paracoccus aminophilus* JCM 7686 as a case study

Elvira Chapkauskaitse, *University of Warsaw (PL)*

16:25–17:25 **Oral session: Plan(t)s for the future – Plant biotechnology**

O-19 Immobilization of *Rindera graeca* transgenic roots on constructs made of biodegradable polymers

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

O-20 Knocking-out of gene/s encoding acyl-CoA:lysophosphatidylethanolamine acyltransferases inhibit plant senescence

Sylwia Klińska, *University of Gdańsk/Medical University of Gdańsk (PL)*

O-21 Is lutein required for healthy deetiolation of angiosperms? Greening of *lut2* Arabidopsis mutants

Kamil F. Trzebuniak, *Jagiellonian University, Kraków (PL)*

17:25–18:00 **Break**

18:00–18:45 **Workshop**

W-1 How to publish your article?

Jan Cools, *KU Leuven (BE)*

Sunday, the 23rd of May 2021.

10:00–11:00 **Oral session: What goes around comes around – Environmental biotechnology**

O-22 Specific microbiome signatures under the canopy of Mediterranean shrubs

Mohamed Idbella, *Federico II University of Naples (IT)*

O-23 Identification of selected *mer* operon genes in bacteria isolated from *Tussilago farfara* L. microbiome growing in mercury-contaminated and non-mercury-contaminated areas

MagdalenaTrojańska, *Jagiellonian University, Kraków (PL)*

O-24 Bio-inspired activation of carbon-halogen bonds in aqueous microheterogeneous solutions

Joanna Szczepanik, *Institute of Organic Chemistry Polish Academy of Sciences, Warsaw (PL)*

O-25 Comparison of pheno- and genotypic properties of *Listeria monocytogenes* strains isolated from clinical samples and from the environment

Katarzyna Grudlewska-Buda, *Collegium Medicum UMK, Bydgoszcz (PL)*

10:00–11:00 **Oral session: Crisper than CRISPR – Molecular biology**

O-26 Half way to hypusine. Structural analysis of human deoxyhypusine synthase

ELŻBIETA WĄTOR, *Jagiellonian University, Kraków (PL)*

O-27 DNA damaging effect of ATR and CHK1 inhibitors combined with olaparib in HR deficient and proficient high grade serous ovarian cancer cell lines

PATRYCJAGRALEWSKA, *University of Łódź (PL)*

O-28 Cloning and overproduction of the potential lipase Lip628 from the *Psychrobacter cryohalolentis* 11E8b strain in the *Pichia pastoris* system

KINGA GLUCHOWSKA, *Institute of Physical Chemistry Polish Academy of Sciences, Warsaw (PL)*

11:00–11:10 **Break**

11:10–12:05 **Plenary lecture**

PL-6 Summer with mosquitoes and ticks, and then? Babesiosis, borreliosis and dirofilariasis

ANNA BAJER, *University of Warsaw (PL)*

12:05–12:15 **Break**

12:15–13:15 **Poster session 2 (P-39–P-76)**

13:15–15:00 **Lunch break**

15:00–15:55 **Plenary lecture**

PL-7 Application of Machine Learning/ Deep Learning/Artificial Intelligence to Drug Discovery

PHILIP TAGARI, *Amgen Inc. (US)*

16:00–16:30 **Closing**

Poster Session 1

Saturday, the 22nd of May 2021, 14:15–15:15

P-1 Substrate specificity of acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT) from microalgae *Phaeodactylum tricornutum*

Ada Połońska, *University of Gdańsk/Medical University of Gdańsk (PL)*

P-2 Scent of quality – microorganisms and volatile metabolites in aerobically and MAP packed poultry meat

Adam Szymajda, *Łódź University of Technology (PL)*

P-3 Natural pigments in microalgae – their extraction and separation

Adrianna Maria Zalewska, *Warsaw University of Technology (PL)*

P-4 Ibuprofen can modulate MSCs ability to promote macrophages polarization into M2 phenotype

Agnieszka Kulesza, *Medical University of Warsaw (PL)*

P-5 Reduction of carboxylic acid catalysed by W-dependent aldehyde oxidoreductase from *Aromatoleum aromaticum*

Agnieszka Winiarska, *Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, Kraków (PL)*

P-6 Multiomics analysis of the driver's oncogenes function in human cancer

Akanksha Jaiswar, *Mossakowski Medical Research Institute Polish Academy of Sciences, Warsaw (PL)*

P-7 Stressed out: analyses of polyphosphate levels in *Escherichia coli* during different stress conditions

Aldona Wierzbicka, *University of Gdańsk/Medical University of Gdańsk (PL)*

P-8 Enzyme coimmobilization on silica monoliths

AleksandraŁochowicz, *Silesian University of Technology, Gliwice (PL)*

P-9 Bacteria inside a lichen? A case study of the Antarctic lichen *Leptogium puberulum* microbiome

Aleksandra Woltyńska, *Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw (PL)*

P-10 The effect of brominated flame retardants on selected apoptotic parameters of human peripheral blood mononuclear cells

Anna Włuka, *University of Łódź (PL)*

P-11 Disruption of glycosomal protein import: sweet death of trypanosoma

Artur Blat, *Jagiellonian University, Kraków (PL)*

P-12 Application of fluorescence *in situ* hybridization technique to visualize choosed chromosomes in domestic cat oocytes

Barbara Kij, *University of Agriculture in Kraków (PL)*

P-13 Photocatalytic and microbial activity of titanium(IV) oxo-complexes in PMMA matrix composites

Barbara Kubiak, *Nicolaus Copernicus University, Toruń (PL)*

P-14 Light my antibiotic resistance. A new chance to resensitize multidrug resistant strains of *Enterococcus* sp. to antimicrobials

Beata Kruszewska, *University of Gdańsk/Medical University of Gdańsk (PL)*

P-15 Identification of kinases that phosphorylate Gli proteins

BrygidaBaran, *University of Warsaw (PL)*

P-16 Influence of the rotating magnetic field on the diffusion process of antibiotics

Daria Ciecholewska-Juśko, *West Pomeranian University of Technology, Szczecin (PL)*

P-17 Novel gain-of-function mutations R249C and S250C in complement C2 protein identified in patients suffering from rare kidney diseases

Daria Kowalska, *University of Gdańsk/Medical University of Gdańsk (PL)*

P-18 Immobilized multi-enzymatic cascade for L-erythrulose production

Daria Świętochowska, *Silesian University of Technology, Gliwice (PL)*

P-19 Enzymatic chiral cyanohydrins synthesis in continuous flow microreactor

Dominika Stradomska, *Silesian University of Technology, Gliwice (PL)*

P-20 Innovative cosmetic formulations with microalgae extract – preparation and testing

Dominika Woźniak, *Warsaw University of Technology (PL)*

P-21 P-solubilizing *Streptomyces roseocinereus* MS1B15 with multiple plant growth promotion increased barley plant growth, and soil nutrients

Fatima Ezzahra Chouyia, *Federico II University of Naples (IT)*

P-22 Analysis of *OGG1* and *MUTYH* gene expression in two brain regions of rats subjected to chronic mild stress and during escitalopram drug intake

Gabriela Barszczewska, *University of Łódź (PL)*

P-23 Analysis of gene expression promoting proliferation in squamous colorectal cancer

Hanna Cieślak, *Stefan Wyszyński University, Warsaw (PL)*

P-24 Isolation of exosomes excreted by SW480 cancer cells and their preparation for drugs loading

Hubert Grel, *Warsaw University of Life Sciences (PL)*

P-25 Evaluation of platinum nanoparticles-doxorubicin interactions

Inez Mruk, *University of Gdańsk/Medical University of Gdańsk (PL)*

P-26 Production and characterization of monoclonal antibodies specific to different isoforms of bacteriocin BacSp222 using hybridoma technology

Jacek Litewka, *Jagiellonian University, Kraków (PL)*

P-27 Does light exposure trigger pheomelanin production in fungi? – EPR studies

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

P-28 Sewage as a rich source for isolation of host-specific bacteriophages against bacteria from *Enterobacteriaceae* family

Joanna Pitucha, *West Pomeranian University of Technology, Szczecin (PL)*

P-29 Develop new inhibitors of II type secretion system from *Pseudomonas aeroginosa*

Jordan Sycz, *Wroclaw University of Environmental and Life Sciences (PL)*

P-30 Analysis of copy number of selected chromosomes in hybrid embryos *(Bos taurus* × *Felis catus)*

Julia Gabryś, *University of Agriculture, Kraków (PL)*

P-31 Pipeline for diversity assessment of eukaryotic rhodopsins in metatranscriptomic data

Julia Gołębiowska, *University of Warsaw (PL)*

P-32 Western diet (WD) may trigger brain changes typical for Alzheimer's-like neurodegeneration

Justyna Domańska, *Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw (PL)*

P-33 The newly discovered, ammonium-tolerant microalga *Chlorella vulgaris* K-01 and its superpowers

Justyna Kowalczyk, *Jagiellonian University, Kraków*

P-34 Altered metabolism of auxin in *Arabidopsis thaliana* under ammonium nutrition

Kacper Dziewit, *University of Warsaw (PL)*

P-35 High concentrations of saturated and polyunsaturated fatty acids regulate the activity of autophagy in hypertrophic adipocytes

Karolina Ciesielska, *Warsaw University of Life Sciences (PL)*

P-36 Electrochemically controlled modification of polyamide with nanoparticles of antibacterial properties

Karolina Kowalewska, *University of Łódź (PL)*

P-37 Miniaturization of polarized liquid–liquid interface as a powerful tool in quinine determination in real samples

Karolina Sobczak, *University of Łódź (PL)*

P-38 Expression of *IKBKB* in peripheral blood and brain regions of rats subjected to chronic mild stress and venlafaxine treatment

Katarzyna Białek, *University of Łódź (PL)*

Poster session 2

Sunday, the 23rd of May 2021, 12.15–13.15

P-39 *In vivo* analysis of antimicrobial activity of phages infecting *Pseudomonas* spp., *Serratia* spp. and *Aeromonas* spp.

Katarzyna Bujak, *University of Warsaw (PL)*

P-40 The application of MALDI MSI technique in the evaluation of symbiosis and pathogenesis in plants

Katarzyna Suśniak, *Maria Curie-Skłodowska University, Lublin (PL)*

P-41 The effect of polystyrene nanoparticles of different diameters on DNA damage in peripheral blood mononuclear cells

Kinga Kik, *University of Łódź (PL)*

P-42 Cyclosporine A changes expression of TGFβ1-3 in keratinocytes treated with LPS

Magdalena Gradzik, *Katowice School of Technology (PL)*

P-43 Effect of the rotating magnetic field on bacterial biofilm extracellular matrix physical and chemical properties

Magdalena Szymańska, *West Pomeranian University of Technology, Szczecin (PL)*

P-44 18S rDNA amplicon-based analysis of the diversity of microbial eukaryotes' communities in the salinity gradient of Świna river estuary

Małgorzata Chwalińska, *University of Warsaw (PL)*

P-45 Structure-function analysis of anti-viral protein IFITM3

Małgorzata Tyrakowska, *University of Gdańsk/Medical University of Gdańsk (PL)*

P-46 Isolation and identification of microorganisms able to degrade the xenobiotics

Marianna Wyszomirska, *Warsaw University of Technology (PL)*

P-47 Impact of chronic myeloid leukemia environment on the level of DNA-double stand break and activation of repair pathways

Marta Jakubik, *Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw (PL)*

P-48 Selection of small molecule DDB1 ligands

Marta Słowianek, *Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Łódź (PL)*

P-49 Activity of inhibitors of the two *Helicobacter pylori* purine salvage pathway enzymes (purine nucleoside phosphorylase – PNP and adenylosuccinate synthetase – AdSS) *in vitro* and *in vivo*

Marta Ilona Wojtyś, *University of Warsaw (PL)*

P-50 Differentiated murine Neuro-2a as a model to study toxicity of metal nanoparticles

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

P-51 LSD1 activity promotes LPS-induced inflammation of microvascular endothelial cells

Martyna Wojtala, *University of Łódź (PL)*

P-52 Effect of cysteine mutations on the function of the mitochondrial form of the peroxide sensor Gpx3

Mateusz Bałka, *University of Glasgow (UK)*

P-53 YEAST, WE CAN! The Factory of Extracellular Cellulases for Biotech Applications

Mateusz Młynek, *Warsaw University of Technology (PL)*

P-54 Identification of a novel Ag-binding peptide and its presentation on the surface of T7 phage capsid

Mateusz Szymczak, *University of Warsaw (PL)*

P-55 Impact of copper ions addition on *Miscanthus* × *giganteus* plant regeneration via somatic embryogenesis in callus obtained from immature inflorescences

Gabriela Belniak, *Adam Mickiewicz University, Poznań (PL)*

P-56 The potato juice as cost-effective medium for biosynthesis of bacterial cellulose

Michał Broda, *West Pomeranian University of Technology, Szczecin (PL)*

P-57 Evaluation of the most efficient isolation method of mesenchymal stem cells (MSCs) from different regions of human umbilical cord for medical treatments

Monika Sypecka, *University of Warsaw (PL)*

P-58 New bacteriophages infecting *Bacillus* spp.

Natalia Kielich, *Adam Mickiewicz University, Poznań (PL)*

P-59 Fungal battle – pathogenic versus endophytic fungi of ash (*Fraxinus excelsior* L.) in a study on active substances – the weapon production by the endophytes

Natalia Marcol, *Jagiellonian University, Kraków (PL)*

P-60 Optimization of process of cloning and expression of the ice-binding protein isolated from psychrophilic yeast *Glaciozyma*

Natalia Rutkowska, *Łódź University of Technology (PL)*

P-61 Platinum nanoparticles and cisplatin: interactions and biological activity of the analyzed substances

Patrycja Bełdzińska, *University of Gdańsk/Medical University of Gdańsk (PL)*

P-62 Visualization of actin cytoskeleton dynamics, analysis of cell cycle progression and proliferation in dermal fibroblasts derived from wild cats – preliminary results

Patrycja Mrowiec, *University of Agriculture in Kraków (PL)*

P-63 Targeting proteins for degradation in bacteria: a new approach for antibiotic discovery and functional study of protein functions

Patrycja Szybowska, *University of Warsaw (PL)*

P-64 Colocalization of New Coumarin-based Fluorescent Probes Using LysoTracker Red and MitoTracker Red probes

Patrycja Środa, *Cracow University of Technology, Kraków (PL)*

P-65 Inexpensive and simple ways to miniaturize electrified liquid/liquid interface

Paulina Borgul, *University of Łódź*

P-66 Optimizing the multi-cell isolation process to approach the real-life interactions of skin cells *in vitro*

Paulina Musolf, *Warsaw University of Technology (PL)*

P-67 The application of ion transfer voltammetry in determination of phenylethylamine in milk samples

Robert Karpiński, *University of Łódź (PL)*

P-68 Antimicrobial potential of St. John's wort plant extracts to various test microorganisms

Sandra Smilevska Stevkovska, *Ss. Cyril&Methodius University, Skopje (MK)*

P-69 We can't use fluorescein diacetate for diatom viability assessment! Or can we?

Stanisław Listwan, *Jagiellonian University, Kraków (PL)*

P-70 Research on cryptophycin production by cyanobacteria *Nostoc* sp.

Stanisław Mec, *Łódź University of Technology (PL)*

P-71 The role of Ubn2 protein in transcriptional repression of the Hedgehog pathway

Weronika Skarżyńska, *University of Warsaw (PL)*

P-72 Adalimumab changes expression of TGFβ1-3 in keratinocytes treated with LPS

Weronika Wieczorek, *Katowice School of Technology (PL)*

P-73 Boosting with yeast, or how to get diatoms to make more goodies

Wiktor Tokarek, *Jagiellonian University, Kraków (PL)*

P-74 Alone we can do so little, together we can do so much – breast cancer cells and fibroblasts clusters in metastasis process

Wiktoria Martysiewicz, *University of Gdańsk/Medical University of Gdańsk (PL)*

P-75 Effect of IGH enhancers inhibition with compound 30666 on B-cell lymphoma survival and oncogene expression

Wojciech Łosiewski, *Institute of Human Genetics Polish Academy of Sciences/University of Medical Sciences, Poznań (PL)*

P-76 The role of sRNAs molecules in an antirepressor-mediated initiation of the phage lytic cycle

Wojciech Wesołowski, *University of Gdańsk (PL)*

PL-01: More than just a crosstalk, histone marks can be drivers of the changes in splicing observed during the epithelial-to-mesenchymal transition

*Reini Fernandez de Luco**

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Histone marks have long been suggested to play a role in the regulation of alternative splicing. From such studies, the impact of chromatin in splicing seemed limited, affecting just a small number of exons, and in a modulatory way by just fine-tuning the final splicing outcome. By combining genome-wide transcriptomics and epigenomics machine learning analyses, we have found that chromatin modifications can differentially mark a third of all the alternatively spliced cassette exons expressed in human embryonic stem cells in a combinatorial and position-dependent way, creating what we called Splicing-Associated Chromatin Signatures (SACS). These SACS coordinate the splicing of functionally related genes sharing common RNA binding motifs to an extent that changes in splicing between human cell types correlate with changes in the enrichment of these SACS. Even more, a highly localized change in a single histone mark right at the exon of interest, using CRISPR epigenetic editing tools, is sufficient to induce a change in splicing that can recapitulate the epithelial-to-mesenchymal transition. Taken together, we have evidence of the dynamic role of histone marks in driving the alternative splicing of subset of genes with specific functional and/or regulatory characteristics that could be reverted in a therapeutic context, like for inhibiting EMT-dependent cancer metastasis.

Professor Reini Fernandez de Luco received a PhD degree in 2007 in Dr. Jorge Ferrer's laboratory (Hospital Clinic de Barcelona, Spain) conducting research on the role of the transcription factor HNF1a in the nuclear organization of its target genes using *in vivo* mouse models. During her postdoctoral fellowship Professor Reini Fernandez de Luco was interested in the impact of histone marks in alternative splicing process. Since 2013, she has been holding a position of Group Leader at the Human Genetics Institute (IGH-CNRS) in Montpellier (France). Histone marks have long been suggested to play a role in the regulation of alternative splicing. From such studies, the impact of chromatin in splicing seemed limited, affecting a small number of exons in a modulatory way by just fine-tuning the final splicing outcome. By combining genome-wide transcriptomics and epigenomics machine learning analyses with exon-specific CRISPR epigenetic editing, Professor Reini Fernandez de Luco's group has found that histone marks are drivers of the changes in alternative splicing of subset of genes with specific functional and/or regulatory characteristics that could be reverted in a therapeutic context, for example, by inhibiting EMT-dependent cancer metastasis.

PL-02: 3D cell-culture scaffolds generated using microfluidics

*Jan Guzowski**

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Development of efficient and reproducible methods of culturing of fully functional tissues *in vitro* is a bottleneck in tissue engineering and regenerative medicine. A solution to this problem could open way to personalized drug testing or personalized cell therapies for treatment of diseases such as cancer or type 1 diabetes. One of the remaining challenges is the high-throughput formulation of large numbers of copies of an artificial microtissue with the functionality—such as metabolic activity or response to drugs—resembling the native tissue. We address this challenge via encapsulating and culturing cells inside hydrogel microspheres using microfluidics. Hydrogel droplets are generated with extreme reproducibility (polydispersity 1-5%) at a junction of microchannels and subsequently assembled into larger meso-structures with predesigned spatial cell arrangement resembling the native tissue. As a proof of concept we focus on one of the most abundant tissues: vasculature, and we study the development of vascular networks in real time in multiple parallel experiments in reproducible conditions. The results indicate the possibility of using granular hydrogels as precursors of vasculature for applications in modeling of vascular diseases or in biofabrication of various types of vascularized tissue-like constructs.

Dr. Jan Guzowski is a physicist with interests in soft matter science, droplet microfluidics, granular matter and tissue engineering. He is a leader of Soft Granular Matter and Tissue Engineering Group at Institute of Physical Chemistry PAS (IPC PAS) in Warsaw. The focus of the group is structure formation in granular materials, in particular those composed of close-packed monodisperse droplets or hydrogel beads generated using microfluidics, and applications of such structures in tissue engineering, 3D cell culture and organ-on-chip technologies. Dr. Guzowski completed his PhD in Max Planck Institute for Intelligent Systems in Stuttgart, Germany, followed by postdoc positions at IPC PAS in Warsaw with Prof. P. Garstecki and at Princeton University, USA, with Prof. H.A. Stone. He is a laureate of the First Team programme (2017-2021) of the Foundation for Polish Science.

PL-03: The use of lignocellulosic waste materials for treatment of heavy metal containing wastewaters – discussions on effectiveness and profitability

*Łukasz Drewniak**

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Industrial wastewater treatment is usually a cost-intensive process, which means that competition in the treatment technology market focuses not only on the efficiency and effectiveness of the proposed solutions, but also on the reduction of investment and operating costs. In this perspective, a passive bioremediation systems based on the use of lignocellulosic waste materials seem to be a very attractive solution, because they enable efficient wastewater treatment at low cost. In this work, the results of the use of spent mushrooms compost, sawdust and wood chips in industrial wastewater treatment systems containing high concentrations of metals and sulfates will be presented. The details of laboratory and pilot studies will be discussed in the context of the efficiency and profitability.

Łukasz Drewniak, Ph.D, D.Sc., is an Assistant Professor at the Department of Environmental Microbiology and Biotechnology at the Faculty of Biology, University of Warsaw. He specializes in microbiology and environmental biotechnology and has been managing a research team, which conducts projects in the field of the bioremediation of environments contaminated with heavy metals, organic compounds, and the biodegradation of organic waste materials. He completed his Ph.D. in Biological Sciences in 2009 and he obtained degree of D.Sc. in 2017 (habilitation) at the University of Warsaw. He is one of the leaders in the commercialization of research results and inventions developed at the University of Warsaw. He was awarded for implementation activities by the Minister of Science and Higher Education in 2019. He is also a founder and a main shareholder of the first spin-off company of the University of Warsaw – RDLS Sp. z o.o.

PL-04: Gene transfer agents: bacteria tame phage and put them to work

*Andrew Lang**

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Gene transfer agents (GTAs) are bacteriophage-like particles produced by some prokaryotes that exclusively package small fragments of cellular DNA. Production of GTA particles by the alphaproteobacterium *Rhodobacter capsulatus* occurs in a small subset of the population (<3%), with these cells lysing to release particles that can then transfer the packaged DNA to other cells in the population. The production of GTAs in *R. capsulatus* is controlled by multiple cellular regulatory systems and coordinated with the ability of nonproducing cells to become competent to receive DNA from the released GTA particles. My presentation will cover various aspects of GTA biology, from structure and evolution to what is known about the mechanisms for regulating production.

Andrew Lang is a Professor at the Memorial University of Newfoundland in St. John's, Canada. He completed his PhD at the University of British Columbia in 2000. Professor Andrew Lang's current research concerns primarily gene transfer agents. However, his scientific interests cover several aspects of virus biology: from studying the movement of genes among bacteria by viruses, to the movement of genes between strains of viruses through recombination, to the movement of viruses among different host species and geographic regions. In his studies, he combines a variety of approaches, including molecular biology, biochemistry, genomics and bioinformatics.

PL-05: The biogenesis and function of the nucleosome landscape

*Felix Müller-Planitz**

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The Mueller-Planitz lab studies core components of chromatin – the nucleosomes and the machinery that places them in the genome. Nucleosomes are crucial to human health. Aging, for instance, disrupts the nucleosome landscape, destabilizing the genome, and mutations in nucleosomes are drivers of cancers. Nucleosomes serve both as barriers that restrict access to the genome and as a medium to accumulate epigenetic marks. Correspondingly, the locations of nucleosomes in the genome are precisely controlled by so called nucleosome remodeling complexes. Remodelers move, assemble, or eject nucleosomes in an ATP-dependent fashion. Some also even the spacing between nucleosomes, setting a characteristic nucleosome to nucleosome distance. These 'spacing remodelers' thereby generate arrays of nucleosomes with a surprising regularity, and these arrays are conserved throughout eukaryotes. Their function however remains elusive. The overarching aim of the Mueller-Planitz lab is to elucidate the biogenesis of the nucleosome landscape and dissect its biological function. To achieve this goal, his lab bridges methodologies of molecular biology, genetics, genomics, biophysics, structural biology, and enzymology. They develop cutting-edge technology to visualize individual nucleosome patterns in single cells and to dissect the mechanism of nucleosome remodeling genome-wide *in vivo* and *in vitro*.

Felix Müller-Planitz received his Ph.D. from Stanford University for mechanistic work on DNA topoisomerase II using biochemical and biophysical methods, in particular transient kinetic approaches. For his postdoc, he switched fields and joined Prof. Becker's lab at the LMU in Munich, where he got exposed to the world of chromatin that continues to be his home to date.

PL-06: Summer with mosquitoes and ticks, and then? Babesiosis, borreliosis and dirofilariasis

*Anna Bajer**

Department of Eco-epidemiology of Parasitic Diseases, Faculty of Biology, University of Warsaw, Poland

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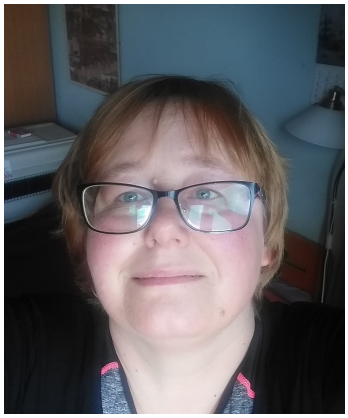
Together with environmental and climatic changes, the geographical range of numerous parasitic diseases has been changing. Interestingly, the most marked changes concern the ranges of vector-borne diseases, transmitted by vectors such as mosquitoes, ticks or fleas.

The aim of this presentation is to introduce the most important diseases vectored by different species of ticks and mosquitoes in Poland. Borreliosis (Lyme disease) is caused by spirochetes of *Borrelia burgdorferi* sensu lato complex. Babesiosis due to *Babesia canis* infection is the most important tick-borne disease of dogs. Dirofilariasis due to *Dirofilaria repens* infection is an emerging disease of dogs and humans in Central and North-Eastern Europe. Recent data on epidemiology, clinical presentation, prophylaxis and control of these diseases will be discussed. I will also present how geographical range of these diseases or incidence changed during the last 20 years in our country.



Anna Bajer is a Head of the Department of Eco-epidemiology of Parasitic Diseases. Her work answers the growing impact of parasitic diseases of environmental and zoonotic origin on human and animal health. In her lab, a range of eco-epidemiological studies is performed on ticks (*Ixodes ricinus*, *Dermacentor reticulatus*, *Haemaphysalis concinna*) and vector-borne diseases (babesiosis, borreliosis, bartonellosis, dirofilariasis); on factors influencing parasite communities in rodents, model free-living hosts (i.e., helminths and haemoparasite community); and on reservoir of intestinal microparasites (*Cryptosporidium*, *Giardia*). She is interested also in molecular diversity and molecular phylogeny of micro- and macroparasites of medical and veterinary significance. Recently, she has also explored vertical route of transmission of vector-borne parasites, from female to offspring, in both environmental and laboratory studies.

PL-07: Application of Machine Learning/Deep Learning/Artificial Intelligence to Drug Discovery

*Philip Tagari**

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The application of Machine Learning (ML)/Deep Learning (DL)/Artificial Intelligence (AI) to speech recognition ("hey Alexa") and image recognition ("deep face") has been extraordinarily successful. These technologies are increasingly being applied to significantly more complex domains including drug discovery. Examples of useful applications including experiment ideation, small molecule and protein, design and bioprocess optimization will be discussed.

Philip Tagari is currently Vice President of Research (Therapeutic Discovery) at Amgen Inc., the world's largest independent biotechnology company. His global laboratories (US, Canada, Germany, China, India) are responsible for biologics discovery, scaffold engineering, optimization and early manufacturability assessment; medicinal, oligonucleotide and peptide chemistry; protein conjugates (Ab-RNAi, peptibodies) and reagents; assay development, screening, enzymological and pharmacological characterization and profiling (*in vitro*), as well as structural biology, biophysics, analytical chemistry, data sciences, materials logistics and automation. His teams have advanced over 30 innovative molecules into clinical development in recent years, including AMG 510 (first-in-class KRASG12C inhibitor) and AMG701 (half-life extended bispecific T-cell engager). Additionally, he is an active member of Amgen Ventures and has participated in numerous research collaborations as well as the integration of Immunex, Tularik, Abgenix, Micromet and Nuevolution into the Amgen laboratories. He is board Chair for Amgen Biopharmaceutical Research and Development (Shanghai) Co., Ltd, a board member of MATWIN (Maturation & Accelerating Translation With Industry) in France and a Director of CQDM (Consortium Quebecois sur la Decouverte du Medicament; Quebec Consortium for Drug Discovery). Prior to joining Amgen in 1998, Philip Tagari was a Research Fellow at Merck Frosst (Canada) Inc., where he contributed to several programs in eicosanoid and inflammatory biology, culminating in the discovery of odanacatib and rofecoxib, as well as the clinically active leukotriene D4 receptor antagonist MK-571 and the leukotriene biosynthesis inhibitor MK-591. Philip is a graduate of Gonville & Caius College, Cambridge University (UK), and performed research at McGill and Oxford Universities on automated image analysis, quantitative immunohistochemistry, and neurotransmitter measurements in neurodegeneration and cerebrovascular research.

I-01: Expanding confocal imaging into the lower magnifications

*Peter Drent**

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Since the introduction of the confocal microscope, 3D imaging has become the norm. By using the confocal.nl RCM upgrade box, a confocal microscope is created on the basis of an imaging microscopy setup, that has 3x better sensitivity, that has 40% better resolution than other (traditional) confocal microscopes on the market. The RCM confocal microscope is very easy to use, offering consistent results for all users.

One of the unique features of the RCM confocal microscope is that it is camera based. Cameras are made with different resolutions, different sensitivities but also with different wavelength sensitivities. Recently new cameras have been released offering 95% QE. These new cameras make the RCM confocal microscope 3x more sensitive that a typical GaAsP PMT (30% QE) based confocal microscope.

With the introduction of RCM2 and its new digital scanning technology, scanning became more precise over the whole field of view. But also the new technology allows full field field of view super resolution scanning, even at 40x with NA of 1.4, resulting in 220 µm x 220 µm field of view, with a final resolution of 120 nm. RCM2 is sensitive because of its camera based detector and the 40x 1.4 is one of the brightest lenses in microscopy, imaging can be done now at only 10 NANO watts of laser light!

Keywords: confocal imaging, re-scanning, near infrared imaging

W-01: How to publish your article?

*Jan Cools**

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Performing good research is what you should aim for, but getting your beautiful data published is still another difficult task. In this workshop, I will highlight the important steps in the publication process. I will discuss the steps to follow to prepare a strong publication and how to deal with reviewers comments and possible rejection. There is a growing number of open access journals and publishing your work in an open access journal has several benefits. Finally, we can also discuss ethical aspects, authorship issues and scientific fraud.

Jan Cools obtained his PhD degree in 2001 from the KU Leuven for a study on chromosomal defects in leukemia. From 2001 to 2003 he continued his research on the genetic causes of leukemia at Harvard Medical School (Boston, USA). After his return to Belgium, he was promoted to full professor in 2009 at KU Leuven. Jan is also a group leader at VIB since 2008. His research team studies the genetic complexity of acute lymphoblastic leukemia (ALL) and uses that information to develop novel models of leukemia and novel treatment strategies. Jan has served as a board member of the European Hematology Association and was the editor-in-chief of the open access journal Haematologica from 2012 to 2017. He is currently editor-in-chief of HemaSphere, a new open access hematology journal of the European Hematology Association.

O-01: The investigation of the T regulatory cells phenotype in chronic lymphocytic leukemia

Marta Śledź[*,1], *Agnieszka Góral*[2], *Angelika Muchowicz*[2]

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Chronic lymphocytic leukemia (CLL), the malignancy of mature B lymphocytes, is the most common type of leukemia in adults. Usually, the CLL progression is slow and patients required no treatment for a long time. In some cases, however, the disease develops into aggressive form, resistant to the therapy. An important role in the CLL progression, is played by cells creating the leukemic microenvironment, including T regulatory cells (Tregs).

The main role of CD4+ FoxP3+ Tregs is an induction of the tolerance for autoantigens. Tregs develop in thymus from autoreactive T cells or in the periphery as the result of T cell polarisation. An increase in the percentage of Tregs, observed in the blood of CLL patients, correlates with the poor prognosis. Therefore it is postulated, that Tregs may be involved in the suppression of anti-leukemic immune response. The aim of this study was to evaluate the phenotype of Tregs in order to understand more deeply the role and function of Tregs in CLL.

To analyze the Treg phenotype, blood samples were collected from patients diagnosed with CLL in the Institute of Hematology and Transfusion Medicine in Warsaw. Peripheral blood mononuclear cells from the patients and healthy donors were isolated and stained with monoclonal antibodies according to the manufacturer's instructions. The phenotype of Tregs was assessed by investigating the expression of markers: CD45RA, CD44, LAG-3, CD69, CCR4, and CD161.

The obtained results confirmed that in comparison to healthy controls, in analysed CLL samples the percentage of Tregs is elevated. Moreover, the analysis revealed the effector phenotype of Tregs collected from these patients. The results may suggest that CLL-associated Tregs function as the suppressors of anti-leukemic immune response, and thereby indirectly support the survival of leukemic cells. The role of Tregs in the CLL progression might be significant and requires further investigation.

Keywords: CLL, Treg, leukemia, flow cytometry

O-02: Acquisition of complement-dependent cytotoxicity by type II anti-CD20 therapeutic antibody obinutuzumab

Alicja Kuźniewska[*,1], *Alan Majeranowski*[1], *Aleksandra Urban*[1], *Sara Henry*[1], *Daria Kowalska*[1], *Marcin Okrój*[#,1]

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Anti-CD20 monoclonal antibodies (mAbs) are widely used in clinics for the treatment of B-cell lymphomas and leukemias. Based on their prevalent effector mechanism, anti-CD20 mAbs are divided into type I (*e.g.* rituximab, ofatumumab) and type II (*e.g.* obinutuzumab) specimens. The first category efficiently activates complement and poorly induces direct cell death, whereas the latter category has the opposite characteristics. These differences were recently explained by structural studies on mAb-antigen complex, which found that type I mAbs bind in a way that favors the recruitment of additional mAb particles and eventually leads to the formation of hexamers, which ideally fits the first component of the classical complement pathway, C1q. For instance, binding of the first particle of type II mAbs precludes oligomerization and results in weak complement engagement. We created a gain-of-function (GOF) variant of complement C2 protein that acts downstream of antigen-mAb complex and C1q binding. C2 is an element of classical complement convertase, which is an enzymatic complex physiologically regulated by endogenous membrane-bound and soluble complement inhibitors. Supplementation of serum with GOF C2 variant and addition of obinutuzumab resulted in elevated convertase activity as well as complement-dependent cytotoxicity of CD20-positive cells, comparable or higher than that observed for rituximab. Our results indicate that obinutuzumab may gain type I characteristics when acts in tandem with hyperactive complement convertases and therefore efficacy of complement activation by type II anti-CD20 mAbs does not rely exclusively on mAb-antigen interaction.

Keywords: complement system, lymphoma, leukemia, obinutuzumab, therapeutic antibodies

O-03: Autophagy inhibition overcomes cisplatin resistance in human lung cancer cells

Aleksandra Olszewska[*,1], *Agata Borkowska*[1], *Claudine Kieda*[2], *Halina Was*[#,2]

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The mainstay of chemotherapy in lung cancer patients is cisplatin and its derivatives, however their effectiveness is unsatisfactory. There are many possible mechanisms of cancer chemoresistance and one of them could be therapy-induced senescence (TIS). TIS cells show an increased activity of the Senescence Associated β-galactosidase (SA-β-gal) and an growth arrest until recently considered as irreversible. However, recent data suggest that TIS cells can re-activate their proliferative potential and lead to cancer recurrence. It might be related with autophagy modulation. Autophagy is a catabolic process, that augments resistance to stressful agents (*e.g.* chemotherapeutics, starvation, hypoxia) and enhances cell survival. Therefore, the aim of our research was to investigate the role of senescence and autophagy in the chemoresistance of lung cancer cells in response to cisplatin treatment. For this purpose, A549 lung cancer cells were treated with cisplatin and autophagy was inhibited using hydroxychloroquine. Additionally, experiments were performed under normoxic ($\sim$19%) or hypoxic (1%) conditions, as hypoxia might significantly change the response to treatment. Our results showed that cisplatin induced senescence of lung cancer cells under both oxygen tension conditions. However, numbers of SA-β-gal positive cells were lower in hypoxia than in normoxia. Additionally, proliferation rate and expression of proliferation-related proteins were increased under hypoxic conditions. Inhibition of autophagy by hydroxychloroquine significantly reduced escaping from senescence.

Altogether, our data demonstrate that hypoxia enhances escaping from senescence after cisplatin treatment what can be overcome with hydroxychloroquine. In order to examine this phenomenon further, animal studies and analysis of clinical samples will be performed. We believe that our studies will help to find a new mechanism of cancer chemoresistance and propose new tools to overcome it.

Keywords: cancer, chemoresistance, autophagy, hypoxia

O-04: The impact of STING activation on neutrophils (Ly6G+ cells) infiltration in two different cancer models

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Stimulator of interferon genes (STING) is one of the components of innate immune response. Its activation leads to type I INFs and cytokines production. This, apart from protecting host from pathogens infection, can provide anti-tumor response. STING agonist (2'3'-cGAMP) administration leads to neutrophils infiltration and activation. Neutrophils have diversified functions in tumors, depending on the microenvironment and stage of tumor development. They can be divided into N1-like (anti-tumor) and N2-like (pro-tumor) cells. The aim of the work was to assess neutrophils infiltration in the tumor microenvironment after intratumoral 2'3'-cGAMP administration in two different tumor models. The experiments were conducted on 4T1 murine breast cancer model and B16-F10 murine melanoma model. 2'3'-cGAMP was administered intratumorally in a dose 5 µg/mice. To assess time when neutrophils activation can be observed, luminol sodium salt was administered. The images were taken using IVIS Lumina System. To visualize the presence of neutrophils in tumor tissue, frozen sections were stained with antibody against Ly6G (clone: 1A8) antigen. The number and phenotype of neutrophils were determined by flow cytometry. Activated (N1-like) neutrophils were characterized as CD11b+Ly6G+ICAM+ cells. There is no observed significant difference in abundance of Ly6G+ cells 6h after cGAMP administration in 4T1 tumor model. In B16-F10 tumor model there is observed significant infiltration of neutrophils 24h after cGAMP administration. Flow cytometry analysis indicates significant increase of the percentage of Ly6G+ICAM+ cells 6h after cGAMP administration in 4T1 tumors. In B16-F10 tumors there is observed increase of the percentage of Ly6G+ICAM+ cells 6, 12 and 24h after cGAMP administration. cGAMP-stimulated neutrophils infiltration and activation seems to occur in different time point depending on the tumor model.

The work is a result of the research project no. UMO-2019/35/N/NZ5/02506 financed by National Science Center.

Keywords: neutrophils, STING, tumor microenvironment

O-05: Microscopic evaluation of the brushed surface of dental ceramics

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The abrasion potential of dentifrices is mainly assessed in the laboratory for practical, scientific and ethical reasons. The most commonly used evaluation method is based on measuring changes in the dentine surface profile after simulating tooth brushing with dentifrice. Decades ago, a definition of Relative Dentin Abrasion (RDA) was developed and for many it is now the “gold standard" for the level of abrasiveness of abrasive powders and dentifrices. Testing the abrasiveness of dentifrice under laboratory conditions is important for developing new formulations, assessing production quality control, and obtaining a rough estimate of potential clinical abrasion [1].

The aim of the study was to evaluate the effect of brushing on the surface of the restoration made of zirconium oxide covered with glaze, feldspathic ceramics and nanofluroapatite. In the study, brushing was carried out with the use of an Oral-B Vitality electric toothbrush with a Precision clean tip and Elmex Sensitive toothpaste and Colgate Total Whitening toothpaste. Each brushing was performed for 2 minutes with a pressure of 2 N, using 0.2 ml of toothpaste. The surface topography was assessed using a scanning electron microscope (SEM), an atomic force microscope (AFM) in intermittent contact mode before brushing and after 10, 50 brushing cycles. The obtained SEM and AFM images showed that as a result of brushing with a paste with a higher RDA, micro scratches are formed on the surface of the material. Based on the AFM results obtained, the surface roughness parameters were determined.

This research was funded by the National Centre for Research and Development (Warsaw, Poland) within the grant InterChemMed (WND-POWR.03.02.00-00-I029/16-01).

[1] C. González-Cabezas, A. T. Hara, J. Hefferren, F. Lippert, Monogr. *Oral Sci.*, 2013, 23, 100–107.

Keywords: dental ceramics, zirconium oxide, AFM, SEM

O-06: Influence of food components on the gastrointestinal digestion of metallic nanoparticles

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Nowadays nanoparticles are widely used in the food industry. Due to their antimicrobial properties they are often present in the food packaging, they are also added to the food products as colorants or consistence enhancers. On the other hand, the nanoparticle usage in other branches of the industry causes their leakage to the environment and, as a consequence, their absorption to edible plants and animals. Therefore, consumers are constantly exposed to nanoparticle ingestion. According to the current state of knowledge, metallic nanoparticles may appear seriously hazardous for human health, causing cancer or damage to cell organelles. In this context exposure to nanoparticles should be deeply investigated. However, there is still lack of vast examination of nanoparticle fate in human gastrointestinal tract. As nanoparticles are not ingested on their own but rather as an addition to other products, also the influence of different food components on the nanoparticle behaviour during the gastrointestinal digestion process should be taken into account. In the light of the abovementioned, the investigation has been carried out with the aim of assessing the influence of main food components on the metallic nanoparticles fate during the *in vitro* gastrointestinal simulation. In the course of work, copper oxide nanoparticles have been chosen for thorough examination. A food model has been developed and applied. The experiments have been carried out with the usage of single-particle inductively coupled plasma mass spectrometry (SP-ICP-MS) as a quantitative and qualitative analysis tool and of scanning electron microscopy (SEM) as a means of qualitative analysis. Analyses showed that presence of different food components significantly changes behaviour of copper oxide nanoparticles during simulated gastrointestinal digestion. Possible explanations for the influence of main nutrient groups, i.e. for fat, protein, salts, saccharides and vitamins on nanoparticles have been found.

Keywords: nanoparticles, food, SP-ICP-MS, SEM, gastrointestinal digestion

O-07: Rotating magnetic field exposure increases antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* strains

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Methicillin-resistant *Staphylococcus aureus* (MRSA) remains one of the principal resistant bacterial pathogens causing serious healthcare-associated infections. These bacteria are often resistant to several classes of antibiotics which is correlated with numerous therapeutic failures. The use of various types of magnetic fields to enhance activity of antimicrobial agents may be a promising strategy of fighting infections. However, the mechanism standing behind this phenomenon is not yet fully understood.

This study aimed to evaluate the electromagnetic effect caused by the rotating magnetic field (RMF – magnetic field that has moving polarities in which its opposite poles rotate around a central point) on the changes in antibiotic sensitivity of MRSA strains *in vitro*.

The exposure of bacterial cultures to the RMF was carried out using a self-designed set-up containing a generator of RMF, made of a three-phase stator of an induction squirrel cage motor. Antimicrobial susceptibility of selected clinical MRSA strains exposed to the RMF of f = 5–50 Hz was performed according to the EUCAST and CLSI recommendations.

The results showed that, the use of RMF significantly increased the sensitivity of MRSA strains to the used antimicrobials. The observed effect was depended on the RMF frequency as well as the type and concentration of antibiotics. The results of the current study are an important step towards the development of new therapies for infections caused by multidrug-resistant bacteria.

The work was supported by the National Science Centre, Poland (grant no. 2017/27/B/NZ6/02103).

Keywords: MRSA, antibiotics, rotating magnetic field

O-08: In pursue of a crosstalk between vitamin D receptor and fibroblast growth factor receptors in cells of different origin

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Presented hypotheses and results have their origin in a few years of research concerning 1,25-dihydroxyvitamin D (1,25D) and vitamin D receptor (VDR) in acute myeloid leukemia (AML) cell lines. First reports, about the role of this particular compound and this receptor were published in the 1960s. It was reported that 1,25D could induce AML cell line differentiation. How does it happen? 1,25D, additionally to its main function in calcium-phosphate homeostasis, is involved in hematopoietic stem cell maturation, acting on gene expression via VDR, which is a ligand-activated transcription factor. With this background different hypotheses and possibilities have risen. One has to keep in mind that AML is a very diverse disease and every subtype can respond differently to therapies. It has been reflected in how different leukemia cell lines respond to 1,25D - do they differentiate or not. Our team has observed that in KG-1 cell line, representing a subtype of AML (KG-1 cell line) with fusion gene FOP2-FGFR1 1,25D did not induce differentiation as long as the fusion gene was not disrupted. Therefore, we decided to investigate how fibroblast growth factor receptors (FGFRs), fusion genes including this receptor, and downstream signaling pathways influence VDR in the cells. In my paper I present preliminary data obtained using KG-1 and U2-Os (osteosarcoma cell line, often used in VDR studies). We tested the effect of FGFRs, FGFR1 fusion genes and downstream signaling pathways, including STAT proteins on *VDR* expression and function. Obtained results indicate that STAT proteins contribute to downregulating of VDR expression in leukemic cells but not in osteosarcoma. In U2-Os cells, activation of FGFRs downregulated VDR activity, while FGFR1 fusion genes had no significant effects.

Keywords: vitamin D receptor, acute myeloid leukemia, fibroblast growth factor receptors

O-09: Nrf2 role in immune surveillance

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The transcription factor nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf2) belongs to the Cap 'N' Collar (CNC) family that contains a conserved basic leucine zipper (bZIP) structure. Nrf2 is considered one of the major regulators of cellular defense and survival. It activates cellular antioxidant response by inducing the transcription of a wide array of genes that are responsible for the protection against extrinsic and intrinsic insults, including oxidative stress and xenobiotics. The main Nrf2 target genes represent the most important cytoprotective defense system in cell, including genes responsible for glutathione and thioredoxin production and regeneration, NADPH regeneration, heme and iron metabolism, reactive oxygen species (ROS) and xenobiotic detoxification. Although activation of Nrf2 has a protective role against various toxicants and diseases, the prolonged activation has been shown to favor a progression of several types of cancers, such as lung, breast, head and neck, ovarian, and endometrial carcinomas. Though Nrf2 function in mounting an immune response is still unsolved, it is widely accepted that Nrf2 speeds up growth and proliferation of cancer cells and confers chemoresistance. Our results have shown that depletion of Nrf2 decreased MHC class I expression on protein and cell surface level, in normal lung fibroblasts and in non-small cell lung cancer cell line (A549) with functional knockout of Nrf2. Interestingly, this effect was not observed on transcriptional level where the depletion of Nrf2 increased the expression of MHC class I, in both normal lung fibroblasts and A549 cells. It leads to the assumption that Nrf2 can regulate translation of MHC class I molecules, or affect their degradation. Moreover, co-immunoprecipitation and proximity ligation assay (PLA) results have shown direct interaction between Nrf2 and MHC class I molecules. MHC class I molecules play a crucial role in antigen presentation and in triggering the cellular immune responses.

Keywords: Nrf2, immune response, MHC class I molecules

O-10: Role of microRNA in the regulation of estrogen receptors expression in adipose tissue of obese and normal body weight individuals

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Obesity is characterized by the increase of adipose tissue content above 20% of body mass in men and above 30% in women. In the context of obesity-related complications, not only the amount of adipose tissue but also its localization is essential. Visceral (android) obesity is associated with a high risk of metabolic complications, while femoral (gynoid) may be somehow beneficial. This sexual dimorphism in adipose tissue distribution results from the sex steroid hormones action, especially estrogens. Estrogens exert their action via interaction with their nuclear receptors α and β (encoded by ESR1 and ESR2 genes, respectively). Their amount and activity can be regulated by miRNA. This study aimed to investigate how selected miRNA can influence ESR1 and ESR2 expression in visceral and subcutaneous adipose tissues originating from obese individuals before and after weight loss and in the normal-weight subjects. Expression of ESR1 and ESR2, as well as miR-22-3p and miR-205-5p, was measured by a real-time PCR method. We have observed obesity-associated changes in the expression of the genes encoding estrogen receptors and selected microRNA. In obese individuals, ESR1 mRNA correlated negatively with miR-22-3p levels in the subcutaneous adipose tissue, while ESR2 mRNA levels with miR-205-5p in the visceral depot.

Keywords: obesity, adipose tissue, estrogen, estrogen receptor, gene expression

O-11: Evolution of double β-barrel J-domain proteins – major co-chaperones of the Hsp70 systems

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J-domain proteins (JDPs) are obligatory co-chaperones of Hsp70s. JDP/Hsp70 systems play critical roles in protein folding, trafficking, protection against aggregation, refolding and rearrangements of protein complexes. JDPs guide functional specificity of their Hsp70 partners, all JDPs have a J domain responsible for stimulating the Hsp70's ATPase activity. In this study we focus on the JDPs, which in addition to N-terminal J-domain have C-terminal double β-barrel domain; termed double β-barrel JDPs (dβ b-JDPs). This is a major group of JDPs present in bacteria and in all eukaryotic cell compartments. Traditionally, double β-barrel JDPs are classified into A and B classes, based on the presence or absence of zinc binding region. Both types of dβ b-JDPs interact with unfolded substrates, however, only cytosolic type B dβb-JDPs prevent misfolding of amyloids that are to promote Alzheimer and Parkinson disease. Evolutionary origins of eukaryotic type B JDPs and the way in which they acquired their unique functionalities are not well understood.

Using Hidden Markov Model profiles we have identified members of Type A and Type B JDPs from over 650 proteomes. We determined their evolutionary relationships using Bayesian and Maximum likelihood. Obtained phylogeny is incongruent with classification of double β-barrel JDPs into A and B classes, instead we observed their common origin and divergent evolution within cellular compartments.

Our analysis supports that all eukaryotic dβb-JDPs are more closely related to bacterial type A JDP then to type B. Moreover, cytosolic type B JDPs evolved from type A JDPs ancestor via a duplication event, and their origins are independent from other type B JDPs found in endoplasmic reticulum or in bacteria. The knowledge of dβ b-JDPs phylogeny allowed us to reconstruct structural rearrangements that lead to a divergence of type B JDPs in cytosol.

Keywords: molecular evolution, J-domain proteins, amyloids

O-12: Hairy citrullinated *Porphyromonas gingivalis* enhances Toll-like 2 receptor activation

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Porphyromonas gingivalis (*Pg*) is the keystone oral pathogen implicated in development and progression of periodontitis. One of virulence factors of *Pg* is peptidylarginine deiminase (PPAD), an enzyme citrullinating host- and bacterium-derived proteins and peptides. Importantly, PPAD is essential to stimulate the proinflammatory response in host cells and our results suggested citrullinated fimbriae as the vital factor enhancing inflammation. This *Pg* surface appendage works as a ligand for Toll-like Receptor 2 (TLR2). Using a reporter system in transfected cell line overexpressing the TLR2 receptor we investigated other factors involved in the TLR2 activation. Cells were infected with various *Pg* strains and their mutants deficient in different surface proteins. The highest activation of the TLR2 receptor was observed for the ATCC33277 strain, while the W83 and 381 strains elicited a weak response. Catalytic inactivation of PPAD or fimbriae deletion in the ATCC33277 strain caused significant decrease in their ability to activate TLR2-dependent pathway. TLR2 was also activated by outer membrane vesicles (OMV) isolated from ATCC33277 (citrullination dependently) and W83 (citrullination independently) *Pg* strains. Interestingly, treatment with purified ATCC33277 major fimbriae induced TLR2 activation and co-stimulation with *Pg* LPS did not enhance the signal. In conclusion, the presented results imply that major *Pg* virulence factors activating TLR2 are bacterial cell surface proteins citrullinated by PPAD, especially major fimbriae. Furthermore, *Pg* activated TLR2 in the strain-dependent manner, which suggests that type of fimbriae plays the key role in this process.

Keywords: *Porphyromonas gingivalis*, citrullination, PPAD, fimbriae, TLR2

O-13: Oral pathogens synergize with the inflammatory tissue environment to promote gingival fibroblast activation in periodontitis

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Periodontitis is a chronic inflammatory disease of the periodontium caused by microbial imbalance. Pathological changes in periodontitis are driven by a failed attempt of the host immune system to eliminate pathogenic bacteria. It is known how oral pathogens manipulate immune and gingival epithelial cells to maintain chronic inflammation, but their influence on inflammatory responses of gingival fibroblasts (GFs) in the context of the local microenvironment of the inflamed gingival tissue is not fully understand.

We show that infection of primary human GFs with a laboratory strain of *P. gingivalis* prior to or during stimulation with tumor necrosis factor (TNF) caused synergistic increases in the expression and production of IL-6, IL-8, CCL2 and COX2. A similar amplifying effect on TNF-induced GF activation was observed during infection with clinical *P. gingivalis* strains isolated from periodontitis patients or with other oral pathogens, such as *Fusobacterium nucleatum* and *Filifactor alocis*. Importantly, the observed synergy between TNF and oral pathogens has important functional consequences: conditioned media form GFs infected with *P. gingivalis* in the presence of TNF induced significantly higher levels of STAT1 and STAT3 phosphorylation compared to media from cells stimulated with TNF or infected with *P. gingivalis* alone. Mechanistically, direct contact between cells and bacteria was required for sensitization of GFs to TNF-induced activation, indicating that soluble factors released by *P. gingivalis* are not sufficient for full amplification of GF responses. Consistently, *P. gingivalis* mutants lacking expression of fimbriae, which are essential for bacterial adhesion, failed to synergistically promote GF activation.

Collectively, these results demonstrate that oral pathogens synergize with the inflammatory environment of the gingival tissue to amplify GF activation, which may lead to excessive cytokine accumulation in the gingival tissue.

Keywords: Periodontitis, *P.gingivalis*, gingival fibroblasts, inflammatory response, *F.alocis*

O-14: Bacterial cellulose as a carrier for oral drug delivery system

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The oral drug delivery pathway is seen as the most appropriate method due to its ease of application – convenient for both the patient and the doctor, as well as high noninvasiveness. However, it is not uncommon to encounter problems such as: Incorrect delivery time, the controllability and pace of drug release in patient's digestive system or even intolerances to certain additives found in pharmaceutics. Therefore, it is important to ascertain high biocompatibility and optimization of aforementioned medicine carriers.

Bacterial cellulose (BC), produced by some species of bacteria, has found a plethora of applications, including those in the biomedical field, where it is used mainly as dressing for patients with extensive burns or wounds. Recent researches have confirmed that it also possible to immobilize bioactive substances, such as antibiotics, on BC pellicle. The general goal of this project is to evaluate modified BC-based tablets as a carrier for oral drug delivery system, basing on their physicochemical and pharmaceutical properties, as well as biological activity.

Dry, purified and then ground BC produced by *Komagataeibacter xylinus* bacteria was used for the production of tablets. Tablets with varying weights (100 mg–200 mg), composed of BC and certain percental addition (10%, 20%, 30%) of other natural substances commonly used in pharmaceutics (lactose, mannitol, pectin, starch, alginate). Amoxicillin in different concertation was used as a bioactive substance.

So far, the most promising results were shown within the 20% starch additive-based on tablet dissolvability under conditions imitating those of human's digestive system. It has been confirmed that the use of BC as a carrier allows to regulate the release of the antibiotic in dependence on pH. BC based tablets may provide controlled drug release, potentially can be used in therapy of various types of gastrointestinal infections.

Keywords: bacterial cellulose

O-15: Can diabetes medication help in treating periodontitis patients? Inhibitory effect of metformin on osteoclast formation and activity *in vitro*

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It was confirmed that diabetes and periodontitis are associated with each other. Diabetes is established to lead to hyperinflammatory response of periodontal tissue to bacteria challenge. In turn, periodontitis can cause poor glycemic control in patients with diabetes. Metformin is commonly used diabetes drug. Several reports indicated that metformin may be beneficial for periodontitis patients ex. by improving bone status. However, the influence of metformin on bone resorption is undetermined. Periodontal ligament fibroblasts (PDLFs) anchor the teeth in bone and are known to play a role in formation of osteoclasts and thus are appropriate model to study the influence of metformin on osteoclastogenesis.

Here, we examined the effect of metformin on osteoclastogenic potential of PDLF-PBMC co-cultures. In this model PBMC are source of osteoclast precursors (i.e. monocytes) while PDLFs induce osteoclastogenesis. The cells were cultured for 21 days to allow formation of mature osteoclasts. Staining of TRAcP, marker of osteoclasts, revealed that upon metformin treatment number of these cells was significantly reduced. Additionally, we noticed decreased TRAcP activity in PDLF-PBMC co-cultures. We also studied the effect of metformin on monocytes cultured in the presence of RANKL and M-CSF. Metformin significantly decreased the number of formed osteoclasts both on plastic and on bone slices. After 21 days metformin at lower dose reduced the ability of osteoclasts to resorb the bone, whereas no resorption was observed at the highest dose of metformin. qPCR analysis showed the decrease in the expression of osteoclastogenesis markers: RANK, CtsK and DC-STAMP in monocyte monocultures upon metformin treatment.

Collectively, our results show that metformin significantly reduces osteoclast formation both in mono- and co-cultures indicating that application of this drug may improve bone status of periodontitis patients.

Keywords: periodontitis, diabetes, metformin, osteoclastogenesis

O-16: The structural-functional analysis of complexes of plasmid replication initiation protein, Rep, and the ssDNA

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Bacteria despite their own chromosomal DNA, can also possess additional DNA, which are iteron plasmids. Those molecules replicate independently from the chromosomal DNA, using host's replication machinery and two plasmid-encoded elements: origin of replication and replication initiator (Rep protein). Because DNA replication process in prokaryotic and eukaryotic cells are similar, investigating plasmid replication system might contribute to better understanding also eukaryotic one. Moreover, iteron plasmids carry antibiotic resistance genes. Therefore, better understanding mechanism of plasmid replication initiation can result in indication of eventual new targets of antibacterial drugs. First step of the replication initiation process is the origin sequence recognition by replication initiator proteins. The formation of such a nucleoprotein complex causes melting of dsDNA within DNA unwinding element (DUE). Origin opening provides the single-stranded DNA (ssDNA) for helicase, primase and polymerase, so the replisome is assembled and the new DNA molecule can be synthesized. The data concerning DnaA protein, the bacterial chromosome replication initiator, revealed that it binds to the specific sequence of dsDNA (DnaA-boxes) via DBD domain (DNA binding domain) and single-stranded DNA (ssDNA) within DUE region via AAA+ domain (ATPases Associated with diverse Activities). Rep protein instead of DBD and AAA+ domains possess Winged Helix domains (WH), which specifically bind to dsDNA. Our data showed that Rep proteins are able to interact also with ssDNA within DUE region. So far, crystallographic data obtained by our team, as well as bioinformatic analysis allowed us to resolve the structure of RepE-ssDNA complex. We propose amino acid residues of Rep proteins, which may be responsible for the interaction with ssDNA. Using biochemical approaches, I try to define a role of Rep proteins interaction with ssDNA DUE region for iteron plasmid DNA replication.

Keywords: DNA replication, iteron plasmids, Rep proteins

O-17: Assessment of the toxicological effects of copper and titanium dioxide-based nanomaterials on microbial cells

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The demand for comprehensive and innovative science development has led to an increased emphasis on newly emerging technologies, including nanotechnology. It offers interdisciplinary solutions and innovations for current and future worldwide problems through the application of modern nanomaterials. The implementation of nanoscale products and processes into everyday reality has raised concerns in the scientific world about the possible toxic effects of nanomaterials on living organisms and the environment. Despite attempts to assess the hazards associated with the risk of introducing nanostructures into ecosystems and their toxicity to microorganisms, these issues remain inadequately understood. Therefore, the potential antimicrobial activity of metallic nanomaterials (Cu, TiO_2 and Cu/TiO_2) at different concentrations was tested against two model bacteria – *Escherichia coli* and *Bacillus subtilis*, along with selected 11 microbial species in Microbial Assay for Risk Assessment (MARA test). The morphological changes of reference bacterial cells after exposure to nanomaterials were examined using scanning electron microscopy (SEM). Additionally, the response of enzymatic antioxidant defence systems of bacterial cells to nanomaterial stress was evaluated. The results obtained in the toxicological studies clearly indicated the diverse influence of tested nanomaterials on *E. coli* and *B. subtilis* strains, and MARA microbial strains. Similarly, antioxidant enzymes of *E. coli* and *B. subtilis* showed a specific response in the presence of Cu, TiO_2 and Cu/TiO_2, with Cu having the greatest influence on their activity. SEM images clearly indicated characteristic interactions of nanomaterials with bacterial outer layers, altering the morphology of cells. The inhibitory effect of Cu and TiO_2-based nanomaterials on *E. coli* and *B. subtilis* growth and their ecotoxicological effects on the cells demonstrate their future potential use in commercial products as antibacterial additives.

Keywords: antioxidants, bacteria, nanomaterials, toxicity

O-18: Multiple *parS* centromere-like sites in *repABC* replicons: plasmid pAMI4 of *Paracoccus aminophilus* JCM 7686 as a case study

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Partitioning systems enable the stable maintenance of bacterial low-copy-number replicons. These loci, commonly found in chromosomes and plasmids, consist of two genes encoding partition proteins A and B, and a *parS* centromere-like sequence bound by B-type proteins. In plasmids, the partitioning system is often located in close vicinity to the replication system. For example, in the well characterized alphaproteobacterial *repABC* replicons, the partition (*repAB*) and replication (*repC*) genes form a single operon. A replicon with a more complex *repABC* composition was identified in the *Paracoccus aminophilus* plasmid pAMI4 (438 kb). Besides the *repABC* operon (with *parS* located between *repB* and *repC*) this plasmid contains a 2-kb-long non-coding locus (11.5 kb downstream of *repC*), carrying three additional *parS* repeats. In this study we examined whether this locus, which hasn't been described in other *repABC* replicons, is involved in pAMI4 maintenance. The *parS* repeats were shown to be bound by partition protein B *in vitro* and to contribute to the partition process *in vivo*. Comparative analysis of available *repABC* replicon sequences revealed that multiplication of *parS* is a common phenomenon in pAMI4-related plasmids. Many of these *parS* repeats are located inside protein coding sequences, with an interesting example being the *cas* genes of CRIPSR-Cas systems.

Keywords: plasmid, repABC, partitioning system, Alphaproteobacteria, stable plasmid maintenance

O-19: Immobilization of *Rindera graeca* transgenic roots on constructs made of biodegradable polymers

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Due to the fact that ca. 75% of anticancer drugs originate from plant metabolites, the pharmaceutical industry consider plant cultures as attractive and efficient methods of obtaining such bioactive compounds. Application of modern bioengineering techniques, such as *in vitro* cultures of transgenic (i.e. hairy) roots, provides the possibility to maintain plant biomass with plentiful options of parameters optimization, allowing to exceed natural productivity observed in wild plants. Biomass immobilization is a widely recognized technique of intensifying production of various substances and it is commonly used in scaling-up of *invitro* cultures of plant cells, tissues or organs. Such technique provides a favorable microenvironment for enhanced plant biomass proliferation by supporting resistance of the fragile transgenic roots from hydrodynamic shear forces occurred in agitated culture system.

Basic aim of the study was to investigate the proliferation of *Rindera graeca* transgenic roots if cultured on few biodegradable polymer constructs. Transgenic roots were maintained for 28 days in independent culture systems: non-immobilized biomass (as reference), and as biomass immobilized on three biodegradable polymer-based constructs varied in surface properties.

The most robust proliferation of *Rindera gracea* hairy roots resulted with the highest yield of fresh biomass, i.e. over 120% of biomass has been detected for polymer-supported culture system in comparison to the non-immobilized system. Also the highest level of representative plant secondary metabolite (i.e. 93.7 µg) has been observed for bioprocess supported with biomass immobilization on polymer-based constructs.

Keywords: bioprocess intensification, plant secondary metabolites, transgenic (hairy) roots, biopolymers

O-20: Knocking-out of gene/s encoding acyl-CoA:lysophosphatidylethanolamine acyltransferases inhibit plant senescence

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Autophagy is a biological recycling program, which can delay aging of cells by effective elimination of damaged and potentially harmful organelles or through the degradation of intracellular protein aggregates. In plants this process is not only responsible for life extension but also for surviving in adverse conditions. Latest autophagy reports show that the level of phosphatidylethanolamine (PE), second most abundant phospholipids in plant cell, may affect this process. This phospholipid is necessary in formation of autophagosome via conjugation to ATG8, one of the most important autophagy related protein. Therefore, we decided to investigate the role of two *Arabidopsis thaliana* genes, which encode acyl-CoA:lysophosphatidylethanolamine acyltransferases (LPEATs), enzymes involved in PE synthesis; At1g80950 (encoding AtLPEAT1) and At2g45670 (encoding AtLPEAT2) in plant development.

In our study we determined that knockout lines (lpeat1, lpeat2 and lpeat1 lpeat2) possessed significantly lower level of PE, and elevated level of lysophosphatidylethanolamine. In addition, the autophagy intensity in all knockout lines was inhibited. The NBR1 protein level was higher than in the control (indicated slower autophagic flux) and ATG8 protein level lower than in wild type plants. However, the relative amounts of ATG8 forms did not differed in tested lines. The lpeat mutants exhibited characteristic development pattern with a prolonged lifespan, especially in the rosette senescence time - time from first yellow leaf till complete rosette senescence. Extended lifespan was also observed in other characteristic plant development signs like flowering time, time from sowing to flowering cessation or time between sowing and the time of total yellow leaves appearance. Prolonged lifespan of lpeat mutants (with lower intensity of autophagy) compared to wild type plants was a surprising result, as according to the literature data we should expected shorter lifespan of these mutants.

Keywords: autophagy, *Arabidopsis thaliana*, senescence, acyl-CoA:lysophosphatidylethanolamine acyltransferases

O-21: Is lutein required for healthy deetiolation of angiosperms? Greening of *lut2 Arabidopsis* mutants

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Emerging angiosperm seedlings undergo greening to develop a mature photosynthetic apparatus. During the process etioplasts undergo a transformation leading to chloroplasts formation. The plastid membranes are rearranged, prolamellar bodies are dissolved, and thylakoids and grana are formed. The development of photosynthetic membranes also includes the biosynthesis and assembly of pigments and proteins into photosynthetic complexes. We have compared greening of 7-day old *Arabidopsis thaliana* L. Wild Type (WT) and *lut2* (a lutein-lacking mutant) seedlings. Lutein is the most abundant carotenoid in photosynthetic plant tissue where it contributes to the chlorophyll triplet state quenching.

Etiolated seedlings were illuminated with white light (100 µmol photons $m^{-2}s^{-1}$) for up to 24 hours. We have measured their pigment profile (HPLC and fluorescence), maximal PSII quantum yield (F_V/F_M) and non-photochemical quenching (*in vivo* fluorescence). Plastid ultrastructure (TEM) and the expression of multiple genes (RT-qPCR) were also measured.

Mutant plants overaccumulated xanthophylls (excluding lutein) and were characterized by lower F_V/F_M value than WT plants. There was noticeable difference in non-photochemical quenching kinetics. The transcript level of several genes involved in chlorophyll metabolism and LHC complexes assembly was altered in *lut2* mutants. At the end of the etiolation, the level of the photoactive protochlorophyllide was higher in *lut2* compared to WT. The initial chlorophyllide production was also significantly higher in *lut2* plants. Most surprisingly after 24-hours of deetiolation, prolamellar bodies (characteristic for etiolated seedlings) were still present in *lut2*. In this work we explore the possible causes and implications of the delayed photosystem development based on our experimental data.

Acknowledgements: This work was supported by the National Science Centre, within the Sonata Bis project no. UMO-2013/10/E/NZ3/00748.

Keywords: photosynthesis, lutein, *Arabidopsis*, thylakoid formation

O-22: Specific microbiome signatures under the canopy of Mediterranean shrubs

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Shrub encroachment is a phenomenon in which grasses and herbaceous vegetation are replaced by woody shrubs. The progressive spread of shrubs into grasslands represents a form of land cover change that is widespread in arid and semi-arid grassland ecosystems. Many previous studies have highlighted the effects of shrub encroachment on soil respiration rates and nutrient storage, but little is known about the belowground impacts of this phenomenon. While previous work has considered shrubs to be non-species specific or a single encroached species, we selected six Mediterranean woody shrubs to investigate the effects of their encroachment independently on the soil microbial community. For this reason, the belowground bacterial and fungal communities under the canopy of each shrub and also of the grassland were examined using high-throughput sequencing, coupled with soil and litter chemistry analyses. The results showed a strong independent influence of shrub canopy on bacterial and fungal community diversity, species richness and overall community composition in soil. In contrast to the bacterial community, the fungal community showed high diversity under the shrub canopies. Furthermore, our results showed that shrub encroachment affects soil microbial communities via changes in shrub litter diversity, and thus soil abiotic factors, which thus have assigned a highly specific signature under the canopy of each shrub. For example, the microbial community of grassland and under the *Rosmarinus* canopy was mainly determined by the highest soil Fe content, while the bacterial community of *Pistacia* and *Myrtus* was mainly determined by soil P and Mn content and negatively correlated with soil Cu content. Our results therefore suggest that the individual effect of each shrub on the grassland matrix depends mainly on the chemical properties of the shrub litter, which alters the chemical properties of the soil and thus affects the microbial communities differently.

Keywords: Shrub encroachment, Mediterranean, microbial community, soil chemistry, litter chemistry

O-23: Identification of selected *mer* operon genes in bacteria isolated from *Tussilago farfara* L. microbiome growing in mercury-contaminated and non-mercury-contaminated areas

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One of the most challenging soil pollution is mercury contamination. Some bacteria are able to survive in polluted environments thanks to the resistance mechanism provided by *mer* operon. Mercury resistant bacteria are applied in bioremediation and phytobioremediation.

The aim of this study was to identify selected genes of *mer* operon in bacteria isolated the first time from *Tussilago farfara* L. growing in mercury-contaminated (198.5 ± 10.5 mg Hg/kg dry mass of soil) and non-mercury-contaminated areas (0.058 ± 0.003 mg Hg/kg dry mass of soil). Such research could have great importance when using mentioned bacteria as bioremediators.

The kinetics of bacterial culture growth was studied. Bacteria isolated from *T. farfara* L. growing in mercury-contaminated areas were cultured in standard Luria-Bertani (LB) media with mercury concentration 0,01% (m/v) (Hg source $HgCl_2$ – 135 mg/l media). Isolates from *T. farfara* L. growing in non-mercury-contaminated areas were cultured in standard LB media.

DNA was isolated from a suspension of cells at the logarithmic growth phase. Isolated DNA was characterized by a high degree of purity (A260/A280 1.97) and high concentration 1769.1 ng/µl. To determine the mechanism of the resistance PCR reaction was conducted using primers designed for this purpose. Process led to the identification of chosen *mer* genes in isolated DNA.

Obtained data identified *merA* gene in bacteria isolated from *T. farfara* L. growing in mercury-contaminated soil as well as in several bacteria from uncontaminated environment. *merA* gene encodes mercuric reductase responsible for neutralisation of non-organic toxic mercury forms. Subsequent research will allow to determine the presence of other *mer* genes in both isolates and check whether the *mer* genes are present in chromosomal or plasmid DNA localisation.

Keywords: mercury resistance, *mer* operon, *mer*A, *Tussilago farfara* L., microbiome, bioremediation

O-24: Bio-inspired activation of carbon-halogen bonds in aqueous microheterogeneous solutions

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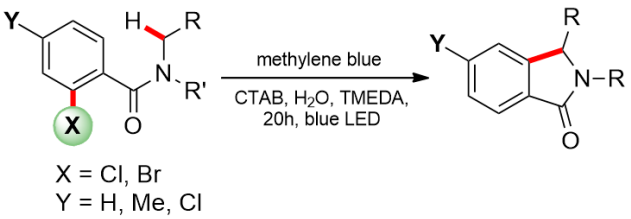
In recent years, much attention has been devoted to methods of site-selective activation of carbon-halogen bonds. Nevertheless, for researchers such reactions of polyhalogenated compounds still remain a challenge. In nature, it be carried out by the activity of enzymes such as iodothyronine selenoprotein deiodinases, iodotyrosine flavoprotein deiodinases, thioredoxin-like dehalogenase, PceA trichloroethene dehalogenase from the *Desulphytobacterium hafniense* strain and bacterial PcpC dehalogenase. However, the effectiveness of photocatalysis in this field is also promising. Nature's ability to convert solar energy into chemical energy in photosynthesis has inspired scientists to develop artificial systems that can mimic this process. Photocatalysis can use cheap and readily available light sources such as LEDs. Thanks to the use of appropriate photocatalysts, it is a new way of transforming various functional groups. This is an ecological approach to chemical synthesis. Light is non-toxic, does not generate waste, and this is important from the point of view of the energy crisis and environmental pollution resulting from human activities. Herein we present the idea of using structured aqueous solutions for the activation of polyhalogenated organic compounds. We investigate the problem of selectivity using halogenated derivatives of benzamide. In the designed setting, two reactions are expected to compete: intramolecular cyclization and dehalogenation. The presented results include optimization of the model cyclization reaction and evaluation of the reactivity of selected, dihalogenated substrates. Our investigations also show interesting mechanistic aspects concerning the radical character of the process.

Keywords: C-X activation, photocatalysis, surfactants, structured solutions



O-25: Comparison of pheno- and genotypic properties of *Listeria monocytogenes* strains isolated from clinical samples and from the environment

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Listeria monocytogenes are Gram-positive, relatively anaerobic, non-spore bacteria that are commonly found in the natural environment, including soil, water, sewage, human and animal feces. The aim of this work was to determine assessment of their genetic similarity, comparison of strains in terms of different properties and compared results regarding ability to form biofilm obtained using different methods among the tested strains of *L. monocytogenes*.

Identification using the MALDI-TOF MS method (Matrix–Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry) and multiplex PCR of 96 strains (isolated from clinical samples (n=58) and from the municipal sewage treatment plant (n=38)) was performed. The genetic similarity of tested strains was determined with the PFGE (Pulsed-Field Gene Electrophoresis) method. To determine the belonging of the tested strains to serological groups (1/2a-3a, 1/2b-3b, 1/2c-3c and 4b-4d-4e) and detect genes encoding selected virulence determinants a PCR multiplex reaction was carried out. The biofilm formation ability of the tested strains on the sterile coupons of AISI 304 stainless steel and polypropylene was examined using the classical culture-based method and ddPCR (digital droplet PCR).

All strains tested belonged to the species *L. monocytogenes* and no genetically identical strains were found. The most numerous was serogroup 1/2a-3a, which included 31 (53.4%) and 32 (84.2%) strains isolated from clinical material and from the sewage treatment plant environment, respectively. Genes *hlyA*, *iap*, *inlA*, *inlB*, *plcA*, *plcB*, *actA*, *prfA* was present in all strains tested. For biofilm formed on the polypropylene and stainless steel, the Pearson correlation coefficient was 0.725 and 0.864, respectively ($p \leq 0.05$). Due to the detection of strains possessing genes encoding virulence factors and forming biofilm on various surfaces, the tested strains of *L. monocytogenes* may pose a potential threat to humans.

Keywords: *Listeria monocytogenes*, serogroup, biofilm formation, virulence gene, ddPCR

O-26: Half way to hypusine. Structural analysis of human deoxyhypusine synthase

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Deoxyhypusine synthase (DHS) is a transferase catalysing the formation of deoxyhypusine, which is the first, rate-limiting step of unique post-translational modification: hypusination. DHS catalyzes the transfer of 4-aminobutyl moiety of spermidine to a specific lysine of eIF5A precursor in an NAD-dependent manner. This modification occurs exclusively on only one protein: eukaryotic initiation factor 5A (eIF5A) and it is essential for cell proliferation. Malfunctions of the hypusination pathway, including those caused by mutations within the DHS encoding gene, are associated with such conditions as cancer or neurodegeneration.

The presented study aimed to investigate substrate specificity of the first step of hypusination using macromolecular crystallography as the main tool and additionally to assess the impact of newly recognized pathological mutations in DHS encoding gene on protein stability, activity and structure.

Human DHS wild type and its two mutants were expressed, purified and crystallized. Our attempts lead to six high-resolution crystal structures of DHS wt in apo form and complexes with natural substrates. Additionally, 2 crystal structures of N173S DHS were determined allowing for detailed analysis and comparison of the apo form and physiologically relevant mutant. Based on crystal structures and activity tests it was shown that despite almost identical binding of spermidine and spermine, probably only spermidine can serve as a proper substrate of deoxyhypusine formation. Furthermore, it was shown that against the previous studies, no conformational changes occur in the DHS structure upon spermidine-binding. Additionally, we proposed four different possible loss-of-function mechanisms for identified pathological DHS variants.

Availability of high-quality structural data will aid the design of novel DHS inhibitors for potential applications in cancer therapy and can significantly advance our understanding of newly recognized genetic DHS deficiency.

Keywords: hypusination, post-translational modifications, neurodegeneration, structural biology

O-27: DNA damaging effect of ATR and CHK1 inhibitors combined with olaparib in HR deficient and proficient high grade serous ovarian cancer cell lines

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Replication stress response (RSR) is characteristic for ovarian cancer, one of the most lethal gynecologic malignancy worldwide. Poly (ADPribose) polymerase inhibitor (PARPi) impairs the repair of single-strand DNA breaks (SSB) in BRCA1/2mut cancers, leading to double--strand DNA breaks (DSB) as a consequence of synthetic lethality. Olaparib (AZD2281) is an FDA approved drug for the treatment of high-grade serous ovarian cancers (HGSOCs), however, resistance to olaparib often occurs. RSR activates DNA repair checkpoint proteins, such as ataxia telangiectasia and Rad3 related protein (ATR) and checkpoint kinase 1 (CHK1). The main objective of the study was to evaluate the genotoxic effect of the new compounds – AZD6738, ATR inhibitor and MK8776, CHK1 inhibitor and their combination with olaparib, on the homologous recombination (HR) deficient and proficient ovarian cancer cell lines – PEO1 (BRCA2mut) and OV90 (TP53mut). For further investigations, compounds concentrations were selected based on the results of the cytotoxic experiments (MTT assay), which established the optimal molar dose for biological effect. The concentrations ratio of 1:1 has been applied in alkaline and neutral version of the comet assay. DNA damage was measured after increasing incubation times (up to 48 h). In the alkaline version of the comet assay, the percentage of the tail DNA is positively correlated with the level of DNA breakage and/or the number of alkali-labile sites, whereas in the neutral version with DSB. Combined treatment led to the accumulation of SSB and DSB in comparison to PARPi monotherapy and significantly changed the comet head and tail morphology. The studies suggest that simultaneous administration of PARPi with ATRi or CHK1i have high genotoxic effect on the BRCAmut as well as TP53mut ovarian cancer cell lines, on the path of synthetic lethality.

This research was funded by the Polish National Science Centre, project grant number: Sonata Bis 2019/34/E/NZ7/00056.

Keywords: ATR inhibitor, CHK1 inhibitor, ovarian cancer, PARP inhibitor, replication stress

O-28: Cloning and overproduction of the potential lipase Lip628 from the *Psychrobacter cryohalolentis* 11E8b strain in the *Pichia pastoris* system

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The main goal of the presented research was the overproduction of potential lipase, with working names Lip628 from the psychrophilic strain *Psychrobacter cryohalolentis* 11E8b isolated from soil collected in the vicinity of the Polish Antarctic station. The great advantage of psychrozymes is the ability to conduct enzymatic reactions at low temperatures, which allows a reduction of the likelihood of thermal degradation of the product during the technological process and a significant reduction of operating costs and energy consumption. After previous failed experiments with *Escherichia coli*, an alternative eukaryotic protein overproduction system has been tried, which is the yeast *Pichia pastoris* system. A vector allowing the secretion of the target protein into the medium (pPICZ αA) and the protease-free strain SMD1168 have been used. The genetic construct pPICZ αA/Lip628 was constructed and then transformed into *P. pastoris* cells by electroporation. The best efficiency of transformation was achieved for HEPES-NaOH (40 mM) and DTT (40 mM) method for frozen cells. Lipolytic activity of the obtained colonies, and therefore the presence of the active form of Lip628 in the genome of *P. pastoris*, were checked, in a functional test, on plates with BMMY medium enriched with 1% tributyrin. Around 6 colonies the presence of halo was observed, indicating the lipolytic activity of the transformants. The presence of the Lip628 lipase coding sequence in the genome of *P. pastoris* was confirmed in 5 of them by PCR. Two recombinants were selected for the overproduction pilot experiment using methanol as the inducer. The overproduction was carried out for a week. Analysis of the collected samples on a polyacrylamide gel showed the presence of an additional band in one of recombinants. The overproduced protein weights of about 36 kDa, but is able to form an oligomer that has a weight of about 130 kDa.

Keywords: lipases, *Pichia pastoris*, protein overproduction

P-01: Substrate specificity of acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT) from microalgae *Phaeodactylum tricornutum*

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The diatom *Phaeodactylum tricornutum* belongs to a major group of photosynthetic oleaginous microalgae producing omega-3 very long chain polyunsaturated fatty acids (VLC-PUFA). Such fatty acids are desired in human diet as they possess both high nutritional and health values.

Acyl-CoA:lysophospholipid acyltransferases (LPLATs) are a group of enzymes found in plants, animals and microorganisms including yeast and microalgae. LPLATs are involved in membrane and storage lipid biosynthesis. In forward reaction they produce phospholipids from lysophospholipids and acyl-CoAs and in reverse reaction produce lysophospholipids and acyl-CoAs from phospholipids and coenzyme A. LPLATs can be divided into LPAAT, LPEAT and LPCAT depending on the preferences towards different fatty acid acceptors. The first group preferentially utilizes lysophosphatidic acid (LPA), the second lysophoshatidylethanolamine (LPE) and the last one lysophosphatidylcholine (LPC). In plants LPCATs plays the most important role in channeling of polyunsaturated fatty acids (PUFA) from the place of their biosynthesis (PC) to triacylglycerols (TAG). So far, LPCATs of microalgae have not been characterized. However, it is expected that they can play a crucial role in the synthesis of omega-3 VLC-PUFA in these organisms by controlling acyl fluxes between PC and acyl-CoA pool.

In the presented studies we have cloned a gene (Phatr3_J20460) of *Phaeodactylum tricornutum* with high homology to the known genes encoding LPCATs. After transformation of yeast lacking ALE1 (the main yeast LPLAT) and preparing microsomal fractions from those yeast, we have performed biochemical assays which proved that this gene is really encoding an enzyme with LPCAT activity. Additionally, we have characterized biochemical properties and substrate specificity of this enzyme.

The research was partially financed by the National Science Centre (NCN), Poland. Project number: 2018/30/Q/NZ3/00497.

Keywords: LPCAT, phosphatidylcholine, omega-3, microalgae, *Phaeodactylum tricornutum*

P-02: Scent of quality – microorganisms and volatile metabolites in aerobically and MAP packed poultry meat

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The poultry meat is one of the most often consumed meat type in Europe. According to European Commission – EU agricultural outlook for markets and income 2019–2030, it is expected that the demand for this type of meat will increase from 25.6 in 2020 to 26.6 kg per person in 2030. Despite the numerous advantages, poultry meat remains sensitive to spoilage, even under refrigerated storage.

Loss of quality in meat products is a complex phenomenon. The activity of microorganisms inhabiting meat results in compounds conversion, which may cause off-odours, changes in taste and other organoleptic features. As consumers recognize poultry meat spoilage first by flavour, volatile by-products of bacterial metabolism appear to be the best early indicators of meat spoilage. The products storage conditions, including composition of gaseous atmosphere and temperature, may also influence on the meat quality.

The aim of the research was microbiological analysis (total viable count, number of *Enterobacteriaceae*, *Pseudomonas* sp., lactic acid bacteria) and SPME-GC-MS-based analysis of the volatile compounds profile during 8 days of refrigerated storage.

Despite initial number of mesophilic bacteria at similar level, their number exceeds 7.0 log cfu/cm^2 on the 5$^{\text{th}}$ storage day of meat packaged aerobically and on 7$^{\text{th}}$ day for meat packaged in a modified atmosphere. The number of *Enterobacteriaceae* in air-packed meat varied from 3.3 log cfu/cm^2 to 6.9 log cfu/cm^2 during storage, when in the meat packed in the modified atmosphere their number was 3.1 log cfu/cm^2 in first day and reached 4.7 log cfu/cm^2 in 5th day. In both type of the meat package an increase of MS signal related to spoilage associated metabolites: isoamyl alcohol and acetoin was observed with strong, positive correlation with total count bacteria and *Enterobacteriaceae*.

Keywords: poultry meat, microbiological quality, SPME-GC-MS

P-03: Natural pigments in microalgae – their extraction and separation

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Microalgae are an extremely interesting and popular subject of research, thanks to their unique chemical composition. The abundance of fatty acids, proteins, vitamins and natural pigments in microalgae, allows for a wide range of their applications in food, pharmaceutical, medical, cosmetic and energy industries. In particular, pigment extracts possess beneficial antioxidant properties. Carotenoids are involved in the cellular defense mechanisms against free radicals caused by ultraviolet radiation and may also be used in anticancer therapies. Chlorophylls seem equally interesting compounds, due to their antioxidant, antimutagenic and antibacterial properties. They can be used in medicine to help wound healing and in food industries as a natural pigment.

The research was carried out to establish an efficient extraction method for natural pigments from three microalgae species: *Dunaliella tertiolecta*, *Cylindrotheca closterium* and *Rhodomonasmaculata*. The pigment extraction process was optimized. Two different mixtures of organic solvents were used to compare the composition of each extracted pigment mix. During the process, three physical approaches to the cell disruption were investigated. The compositions of microalgae extracts were analyzed using spectrophotometry, thin-layer chromatography and high-performance liquid chromatography. A detailed composition of each extract was determined. It has been shown that by selecting a proper mixture of solvents the extraction can be directed toward the desired dye groups. The obtained results confirm that microalgae are a remarkable source of various natural pigments for defined applications.

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Keywords: microalgae, extraction, natural pigments, carotenoids, chlorophylls

P-04: Ibuprofen can modulate MSCs ability to promote macrophages polarization into M2 phenotype

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The role of mesenchymal stromal cells (MSCs) in inhibiting the inflammatory response is determined by their ability to polarize macrophages towards the anti-inflammatory M2 phenotype. It is suggested that the molecule responsible for regulation of this process is prostaglandin E2 (PGE2), which secretion increases in MSCs after activation by proinflammatory inductors. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used to relieve inflammation symptoms. However, their action is based on inhibition of cyclooxygenase-2 (COX-2) - an important enzyme mediating the production of PGE2. Hence the assumption that taking NSAIDs may interfere with natural regenerative processes.

This study investigate whether ibuprofen, the most popular NSAIDs, affects the ability of MSCs to polarise macrophages towards M2.

Primary human monocytes were isolated from buffy coats and differentiated into macrophages (M1 or M2). Cells were cultured in monoculture, or co-culture with primary human bone marrow MSCs. After 24 h cells were treated with ibuprofen (25 µg/ml) for 48h. Additionally, macrophages were treated with supernatants collected after 24h from ibuprofen- treated MSC cultures. Then cells were fixed and stained for surface marker CD206 - characteristic for M2 macrophages.

Study showed 2.9-fold higher expression of CD206 in macrophages cocultured with MSCs compared to macrophages from monocultures. Macrophages from ibuprofen-treated MSC cultures had lower expression of CD206 than in untreated co-cultures (a decrease of CD206+ cells by 19%). In macrophages treated with supernatant from ibuprofen-treated MSC cultures, there was an 1.7 fold increase in CD206 expression compared to untreated control.

The study confirmed the ability of MSCs to polarise macrophages towards M2 phenotype. Results indicate that ibuprofen may interfere with this process, which may impede the reconstruction of damaged tissues.

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Keywords: MSCs, PGE2, ibuprofen, macrophages

P-05: Reduction of carboxylic acid catalysed by W-dependent aldehyde oxidoreductase from *Aromatoleum aromaticum*

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The W-dependent aldehyde oxidoreductases (AOR) are oxygen-sensitive enzymes that catalyse the oxidation of aldehydes to corresponding carboxylic acids. Remarkably, enzymes from the AOR family are also the only known biocatalysts for the respective reverse reaction, reduction of non-activated carboxylic acids to aldehydes at E'≈ –560 mV [1]. The reduction of carboxylic acids can be combined with aldehyde reduction by appropriate alcohol dehydrogenases (ADH) to produce bioalcohol from biomass derived carboxylic acids. This biocatalytic pathway was induced *in vivo* in many microbes (*Caldicellulosiruptor bescii, Moorella thermoacetica, Pyrococcus furiosus*) containing AOR and ADH [2].

The studied AOR from the denitrifying bacterium Aromatoleum aromaticum (AOR*Aa*) is the first aldehyde-oxidizing tungstoenzyme isolated from a mesophilic and facultative anaerobic bacterium. The AOR*Aa* prominent feature is its relatively high resistance to oxygen-inactivation in comparison to other tungsten enzymes. In the cell extract, the enzyme is completely stable, which makes it a good candidate for a catalyst in biotechnological processes. AOR*Aa* is characterized by a broad substrate range, specifically oxidizing aromatic, heterocyclic, aliphatic as well as halogenated aldehydes with benzyl viologen or NAD+ as electron acceptors.

We showed by GC-MS and LC-MS/MS analysis of products, that the reduction of carboxylic acids catalysed by AOR*Aa* is possible in presence of low-potential electron donors. The substrate spectrum of acid reduction is comparable with the reverse reaction. Moreover, we investigated the possible application of the enzyme in the production of various valuable compounds.

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Keywords: oxidoreductases, anaerobic enzymes, carboxylic acid reduction

P-06: Multiomics analysis of the driver's oncogenes function in human cancer

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In cancer, finding the driver mutations is really a difficult task. Till now, the most dependable markers for the status of mutation driver are reoccurrences of mutation in a patient. However, occurrence of mutation varies with each other and that depends upon differences in the endogenous and exogenous mutagens, repair machinery and background mutation rates occurring from different types of infidelity of DNA replication. The High demand to study human cancers leads to novel functional approaches which broaden the scope for therapeutics solutions to upstream and downstream molecular networks of the major oncogenes.

This study mainly focuses on building a systematic, proteomic and transcriptomic map of the main driver oncogenes – cellular proteasome machinery, mutant TP53, KRAS or CMYC, in human cancer which are involved in downstream molecular functions. Here, we have followed a bioinformatics approach including Transcriptomics and Proteomics, to analyze three different cancer types (lung, colon and pancreatic) with three different treatments (mutant TP53, KRAS and CMYC) that includes 12 cell lines. Data quantification has been done by FastQC and trimmomatic tool that resulted 96.47% good quality reads which were further processed for the mapping with HISAT2 tool. In total, 204 up and 4 down common pathways were detected in overlapping with up and down differentially regulated genes. Finally, total 73 core genes including 42 up and 19 down regulated genes common in each cell line have been selected for *in vitro* validation to choose possible therapeutic targets.

Keywords: cancer, oncogene, transcriptomics, proteomics, Differential Gene Expression analysis (DEGs)

P-07: Stressed out: analyses of polyphosphate levels in *Escherichia coli* during different stress conditions

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Polyphosphate (PolyP), is one of the most enigmatic molecules in biology with primordial origin. It is composed of up to 1 000 phospho-anhydride bond-linked phosphate groups, and it is highly conserved in all organisms known. In bacteria, PolyP plays an important role in stress response and virulence and as a phosphate storage, an energy source, a chaperone and as a chelator of metal ions.

During stress conditions, as starvation or heat shock, the stringent response is launched, leading to polyphosphate accumulation, resulting in various stringent factors synthesis, metabolic pathways activation and protein degradation. It all allows the cell to arrest proliferation and to survive through adverse conditions. In *Escherichia coli*, the metabolism of PolyP requires two enzymes: polyphosphate kinase (PPK) and exopolyphosphatase (PPX). It is suggested, that PolyP levels may vary depending on the stress that is applied on the bacteria.

The aim of this research was to implement methods to isolate, measure and compare polyphosphate levels accumulated in bacteria during different stress conditions. The analyses were conducted on *E. coli* MG1655 wild type strain and its isogenic mutants with deletion of genes crucial in PolyP metabolism (Δ*ppk*,Δ*ppx*, Δ*ppk* Δ*ppx*). MOPS minimal medium was used to induce starvation stress and the heat shock was conducted at 45°C. PolyP was isolated from cell lysates using a silica membrane column extraction and it was quantified using enzymatic colorimetric assay. The results show that in both analysed stresses PolyP accumulates to similar amount per cell, but with different synthesis rate. However, the growth rate of cells lacking polyP (Δ*ppk* and Δ*ppk* Δ*ppx)* differs depending on the stress conditions. This work provides broaden knowledge about bacterial stress response and the molecular mechanisms of stress recovery.

Keywords: polyphosphate, stress response, *Escherichia coli*, stringent response

P-08: Enzyme coimmobilization on silica monoliths

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Enzyme immobilization provides heterogeneity of biocatalyst that enables easy separation of the enzyme from solvents and reaction products. Additionally, immobilization increase the thermal and operational stability of enzyme. Many enzymatic reactions require two or even more enzymes, which work together in one cascade and produce the desired compound (product). There are generally three multienzymatic immobilization systems: stepwise, mixed and coimmobilization.

Coimmobilization is now the most common type, in which several kinds of enzymes are immobilized on the same carrier. Because of the close contact of the enzymes the reaction rate and catalytic efficiency are high, and the lag time is eliminated. Coimmobilization is a good solution, when the product of the first enzyme is the substrate for the second one. However, in this type detection of activity and stability is complicated as well as providing the optimal conditions both in the process of immobilization and cascade reaction for each enzyme.

Herein, we examined different variants of coimmobilization on silica monoliths of 3 enzymes: D-amino acid oxidase from *Rhodotorula gracilis*, catalase and transketolase from *Geobacillus stearothermophilus*. Their activity was checked in one-pot cascade of L-erythrulose synthesis.

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Keywords: coimmobilization, multienzymatic cascade, immobilized multienzymatic systems, silica monoliths

P-09: Bacteria inside a lichen? A case study of the Antarctic lichen *Leptogium puberulum* microbiome

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Lichens are a known example of an ideal symbiosis consisting of a heterotrophic fungus, as well as a photobiont, more specifically a green algae or cyanobacteria. However, another component integral to this symbiotic relationship has been recently suggested – a microbiome of heterotrophic prokaryotes. This discovery begs to reassess the current view on the mutualistic relations in the lichen, with the addition of the aforementioned non-photosynthetic microbiome that inhabits the lichen thallus. Here, we tried to investigate the changes in this heterotrophic microbiome depending on the concentration of nutrients in the vicinity of the lichen. The aspects that had been studied in the trophic gradient were as follows: metabolism of carbon sources, total microbial count and bacterial diversity. The difference in the metabolism of the microbial communities had been investigated using the Biolog EcoPlate technique, whereas the total count of the lichen microbiome had been carried out by epifluorescent microscopy. Bacterial diversity had been based on sequence similarities and phylogenetic analyses of the 16S rRNA gene sequences. The eurytopic Antarctic lichen *Leptogium puberulum* was chosen for this study, due to its ability to inhabit both nutrient-rich, as well as nutrient-poor areas of the Point Thomas penguin rookery and Jardine Peak area on King George Island, respectively, allowing for the study of the trophic gradient. Preliminary results show that the microbiome of *Leptogium puberulum* growing near the penguin colony was more abundant and better adapted to metabolising the tested carbon sources, whereas the microbiome of *Leptogium puberulum* growing in the Jardine Peak area was more phylogenetically diverse.

This work was supported by the NCN grant 2017/25/B/NZ8/01915.

Keywords: microbiome, lichen, Antarctica

P-10: The effect of brominated flame retardants on selected apoptotic parameters of human peripheral blood mononuclear cells

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Brominated flame retardants (BFRs) are synthetic substances widely used in industry (manufacture of electrical and electronic equipment, textiles, furniture and other everyday products). Products containing BFRs protect human life and property; however there are fears about harmful impact of these substances. The most commonly used flame retardant is tetrabromobisphenol A (TBBPA). Because TBBPA has been shown to exhibit toxic and possible carcinogenic potential, tetrabromobisphenol S (TBBPS) has been introduced into the market as the substitute of TBBPA. Current research works have not provided a definitive answer to the question of whether or not are TBBPS, 2,4,6-tribromophenol (2,4,6-TBP) and pentabromophenol (PBP) toxic for humans.

The aim of this study was to assess the effect of TBBPA, TBBPS, 2,4,6-TBP and PBP on caspase-9 activity and changes in transmembrane mitochondrial potential in PBMCs. The cells were incubated with the compounds studied in the concentrations ranging from 0,01 to 25 µg/mL for 24 h. Statistical significance was examined on the basis of a comparison of averages using a one-way analysis of variance-ANOVA. To evaluate statistically significant differences between the tested samples, a multiple comparison test - the Tukey test was used.

The results of the study have shown that all BFRs at the concentrations of 25 µg/mL caused statistically significant ($p<0,05$) increase in the caspase-9 activity. It has also been found that TBBPS exhibited the lowest level of caspase-9 activity. PBP caused changes in transmembrane mitochondrial potential from a concentration of 1 µg/mL to 25 µg/mL. The strongest changes were observed under the impact of TBBPA at a concentration of 25 µg/mL.

In conclusion, BFRs increased caspase-9 activity and caused changes in transmembrane mitochondrial potential in human PBMCs at the concentrations that may affect human body during occupational exposure or subacute poisoning with these substances.

Keywords: apoptosis, brominated flame retardants, pbmcs

P-11: Disruption of glycosomal protein import: sweet death of trypanosoma

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Chagas disease is a potentially life-threatening illness caused by protozoan parasite *Trypanosoma cruzi*. Originally it was a neglected tropical disease, but it has spread from endemic countries and currently starts to threaten human health and welfare worldwide. According to World Health Organization (WHO) about 6 to 7 million people worldwide are infected, mostly in Latin America. Only a limited number of treatment options are available they suffer however from toxicity, limited efficacy and increasing resistance. Therefore, an identification of new macromolecular drug targets and a small-molecule modulators is of utmost importance.

The only source of energy in *Trypanosoma* sp. is glycolysis. This process takes place in specialized peroxisomes called glycosomes. The biogenesis of these organelles depends on a number of proteins named Peroxins (Pex). One of them is a cytosolic receptor Peroxin 5 (Pex5), which recognizes peroxisomal targeting signal 1 (PTS1) of proteins that are directed to peroxisomal matrix. In fact, in *Trypanosoma*, the first seven glycolytic enzymes are cargos recognized by Pex5. This carrier-cargo recognition is the first step in protein translocation across the glycosomal membrane.

Recent studies demonstrated that blocking the glycosomal protein import by inhibiting the Pex5 protein carrier, selectively kills *Trypanosoma*. It is caused by the mislocation of glycolytic enzymes to the cytosol, which lack feedback inhibition. This leads to ATP depletion and accumulation of phosphorylated glucose metabolites to the toxic levels, resulting in parasite's cell death.

The aim of this study was to overexpress and purify the *Trypanosoma cruzi* Pex5 for crystallization trials with small-molecule inhibitors. Future goals concentrate on obtaining protein-inhibitor structure for further development and rational design of the Peroxin 5 targeting compounds that may serve as drugs against trypanosomiasis.

Keywords: Chagas disease, glycolysis, glycosomes, peroxins, Pex5

P-12: Application of fluorescence *in situ* hybridization technique to visualize choosed chromosomes in domestic cat oocytes

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The literature shows that abnormalities in the number of chromosomes (aneuploidy) occur more often in oocytes than in spermatozoa, and their formation may be caused by nondisjunction already at the stage of oogenesis. Nondisjunction can conduce to formation of an unbalanced number of chromosomes in daughter cells, leading to embryo mortality or spontaneous abortions. One of the methods of chromosome analysis is the fluorescence *in situ* hybridization (FISH) technique. The FISH technique is used to look for aneuploidy in oocytes of humans, but also of farm animals. Due to the lack of data about chromosomes in domestic cat oocytes, study was conducted to visualize selected chromosomes using fluorescent *in situ* hybridization (FISH).

Ovaries from healthy adult females of domestic cat were obtained after routine ovariohysterectomy. Oocyte-cumulus complexes were possessed from the ovarian cortex and matured *in vitro*. Oocytes with visible polar body were fixed on glass slides. After that, fluorescent *in situ* hybridization with the painting probe specific to the X chromosome was performed. Slides were evaluated using an Axio Imager fluorescence microscope and Zeiss ZEN imager software. Fluorescent signals specific to the feline X chromosome were observed in the domestic cat's oocytes. Our research shows that FISH technique is useful to visualize chromosomes in feline oocytes, which in the future may be used to look for chromosomal abnormalities in these reproductive cells.

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Keywords: oocytes, chromosomes, fluorescence *in situ* hybridization, molecular probes

P-13: Photocatalytic and microbial activity of titanium(IV) oxo-complexes in PMMA matrix composites

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Due to the current epidemic situation in Poland and global spread of the coronavirus around the world, scientists are trying to find a way to stop this virus. Not only viruses are a great threat, but also bacteria, which can also multiply quickly and have a negative impact on the human body. Titanium oxo-complexes may turn out to be a new solution. The aim of the study was the synthesis of $\{Ti_3O\}$ and $\{Ti_4O_2\}$ core (TOCs) as well as the production of composite materials by introducing oxo-complexes into the polymer matrix. The most important step was to test the photocatalytic and microbiological activity of the obtained composites. The obtained compounds can be used in external dressings as well as for the production of antibacterial surfaces.

TOCs were obtained by reacting titanium isopropoxide or isobutoxide with 4-aminobenzoic acid or 4-hydroxybenzoic acid. Composites were obtained by introducing 20% (w/w) oxo-complexes into a polymer matrix. The products were examined by mass spectrometry, infrared spectroscopy, Raman spectroscopy, and the composites were subjected to thermal analysis. The photocatalytic activity was investigated by observing the degradation of methylene blue (MB) and rhodamine B (RhB) under UV and VIS light. Microbiological tests were carried out for the following bacteria: *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* yeasts.

The comparison of MS, IR and Raman spectra allowed to confirm the synthesis of clusters with $\{Ti_3O\}$ and $\{Ti_4O_2\}$ core. The study of the photocatalytic activity and microbiological test confirmed that materials with $\{Ti_4O_2\}$ core exhibit the best properties.

Keywords: titanium(IV) oxo-complex, photocatalytic activity, microbial activity, composites

P-14: Light my antibiotic resistance. A new chance to resensitize multidrug resistant strains of *Enterococcus* sp. to antimicrobials

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For almost 100 years antibiotics have helped with treatment of many bacterial infections. Nowadays, the presence of antimicrobials became so obvious and hardly anyone remembers that before the age of antibiotic therapy most of the bacterial infections were lethal for patients. Deeply disturbing is the fact that the number of newfound antimicrobials is decreasing. Simultaneously, there are more multidrug resistant strains. A new chance for traditional ways of treatment can be antimicrobial photodynamic inactivation (aPDI) which leads to effective bacteria eradication and sensitization to antimicrobials using reactive oxygen radicals during light emission. The current study was aimed to investigate whether combined therapy with clinically used antibiotics and aPDI involving exogenously administered photosensitizer – rose bengal (RB) and green light (λ_{max} = 515 nm) has synergistic effect. In experiments two clinical isolates of *Enterococcus* sp., *E. faecium* and *E. faecalis*, were used. Their drug resistance profile and the synergy between aPDI and antibiotics were characterized with application of different approaches, *i.e.* diffusion assay, MIC evaluation, E-TEST, checkerboard assays, post antibiotic effect. All experiments were performed in accordance with The European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards. Results show that aPDI and RB lead to resensitization of *Enterococcus* sp. to numerous antimicrobial agents: gentamicin, doxycycline, ciprofloxacin and daptomycin. Synergy was confirmed for a few of all employed tests. The possible mechanism of obtained synergistic effects was essayed with specific probes. Singlet Oxygen Sensor Green (SOSG) probes indicated increased production of singlet oxygen in cases of a combination of ciprofloxacin with aPDI against *E. faecalis*. Moreover, investigated synergy can be correlated with the permeabilization of bacterial cell membranes.

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Keywords: antibiotics, resistance, aPDI, *Enterococcus*

P-15: Identification of kinases that phosphorylate Gli proteins

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Posttranslational modifications (PTMs) are key regulatory events for the majority of signaling pathways. In mammalian cells phosphorylation is the most common regulatory PTM and is involved in virtually all major signaling cascades. Transcription factors are often phosphorylated on multiple residues, which regulates their trafficking, stability, or transcriptional activity, depending on the kinase and the site involved. It has been shown that, in addition to the PKA-mediated inhibitory phosphorylation, Gli molecules undergo another phosphorylation event, which is only evident in activated nuclear Gli proteins. Gli proteins are transcriptional effectors of the Hedgehog signaling pathway. In mammals, they are represented by three proteins: Gli1, Gli2 and Gli3. Gli1 acts principally as a transcriptional activator, whereas Gli2 and Gli3 display both activator and repressor functions. They play key roles in the development of many organs and tissues, and are deregulated in birth defects and cancer. It has been proposed that a separate phosphorylation event, independent of PKA, might be required for Gli nuclear translocation, but the site(s) nor the kinase have not been identified. Our research fill this critical gap in our understanding of the regulation of Gli proteins by taking an survey of Gli posttranslational modifications that are triggered by activation. We identified two novel kinases interacting with Gli3 protein. Our research focused on the examination of physical interactions between these kinases and all Gli variants. We also investigated how loss of function of these kinases affect Gli PTMs and thus their activity. This study aims to provide a better understanding of the mechanism of the HH pathway and may lead to the finding of new therapeutic solutions for diseases related to the activity of Gli proteins.

Keywords: Hedgehog, cancer, Gli, kinases, cell signaling.

P-16: Influence of the rotating magnetic field on the diffusion process of antibiotics

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Currently, the numerous strategies for combating the biofilm-forming microorganisms are being developed. Among them, application of the different types of magnetic fields (MFs) have been proposed to boost activity of standard antimicrobial agents. Yet, the mechanisms of MFs action in this context are not fully understood. However, there are indications that antimicrobials in combination with a rotating magnetic field (RMF) may have a higher eradication rate for microorganisms, including biofilm-producing bacteria. This study aimed to explain the electromagnetic effect caused by the RMF on the diffusion process of antibiotics in a solid medium.

The exposure of agar plates with antibiotic discs to the RMF was carried out using a self-designed set-up containing a generator of the RMF (RMF is a magnetic field that has moving polarities in which its opposite poles rotate around a central point), made of a three-phase stator of an induction squirrel cage motor. Measurements were made at several time intervals by cutting out agar discs from which the antibiotics were extracted and their concentrations were determined using the Liquid Chromatography Tandem-Mass Spectrometry (LC-MS/MS) technique.

It was found that RMF can influence diffusion kinetics of antibiotics included in the experiment. However, the amount of antibiotics released from the discs were not significantly affected. Observed effects were depended on the RMF synchronous speed and antimicrobial's type used. The study can help to understand the influence of RMF on the permeability of antimicrobial agents through the biofilm matrix, which could be a breakthrough in the treatment of biofilm infections.

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Keywords: rotating magnetic field, biofilm, antibiotics, diffusion

P-17: Novel gain-of-function mutations R249C and S250C in complement C2 protein identified in patients suffering from rare kidney diseases

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The etiology of rare kidney diseases such as C3 glomerulopathy (C3G) and atypical hemolytic uremic syndrome (aHUS) involves deregulation of the complement system. The common etiologic factor is impairment of proteins that control the alternative complement pathway, as this route is constantly active at a low level and its further propagation depends on endogenous inhibition on self surfaces. Conversely, the classical and lectin complement pathways need specific stimuli such as antibodies and therefore elements of these pathways were not included in routine genetic diagnostics of glomerulopathies. Previously, our group identified the first-ever gain-of-function mutation in the component of the classical/lectin pathway, C2, in aHUS patient. Substitution of serine 250 to cysteine renders classical complement convertase insensitive to regulation by CD55 complement inhibitor and significantly enhance enzymatic activity. However, the driver of pathogenic scenario in gain-of-function (GoF) C2 carriers remained unknown, as the patient was negative for autoantibodies. Herein, we demonstrate one more mutation of the same phenotype, R249C, adjacent to that previously reported, which was found in C3G patient. The presence of either S250C and R249C C2 variants in serum spiked with C-reactive protein (CRP) resulted in the elevated deposition of C3b on the surface of glomerular endothelial cells. To sum up, we confirmed the existence of a mutational hot spot in C2 protein and the potential mechanism, by which elevated acute-phase proteins in GoF C2 carriers may initiate the deposition of complement proteins in kidney microvasculature.

Keywords: complement, C2 protein, aHUS, C3G

P-18: Immobilized multi-enzymatic cascade for L-erythrulose production

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L-erythrulose is a highly valuable chiral building block molecule, important in pharmaceutical and cosmetic industry, which can be synthesized from glycolaldehyde and hydroxypyruvate (HPA) using transketolase (TK). Instead of traditional chemical synthesis of HPA from toxic bromopyruvic acid, this compound can also be synthesized *in situ* by coupling the TK-catalyzed reaction with a D-aminoacid oxidase starting from natural and affordable D-serine. This could also eliminate decomposition of the labile HPA in reaction conditions. It is worth to emphasize, that multi-enzymatic cascade reactions can mediate complicated chemical reactions in one-pot systems, which provides benefits for process design, such as fewer unit operations, improved space-time yields, shorter cycle times, and less waste generation.

However, the application of relatively expensive enzymes imposes the need for multiple use. One of the possibilities to fulfill these requirements is enzyme immobilization, which increases the stability of the enzyme by multipoint interaction with the carrier's surface and protects against molecular interactions. Furthermore, immobilization allows for precise control of the process, i.e. stopping it by separating the biocatalyst from the reaction mixture.

Herein, we propose covalent immobilization of D-aminoacid oxidase from *Rhodotorula gracilis* (DAAO), commercially available katalase (KAT) and transketolase from *Geobacillus stearothermophilus* (TK) on amino modified siliceous pellets and their usage for one-pot, two step L-erythrulose production.

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Keywords: multienzymatic cascade, immobilization, silica carriers, L-erythrulose

P-19: Enzymatic chiral cyanohydrins synthesis in continuous flow microreactor

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Hydroxynitrile lyases (HNLs) are known in biology as part of the plant defence system, where they catalyse the cleavage of hydrogen cyanide (HCN) from cyanohydrins. On the other hand, the reverse reaction is a valid biotechnological process used for chiral cyanohydrins production. Enantiopure cyanohydrins are important building blocks for biologically active compounds, such as pharmaceuticals and agrochemicals [1]. They are synthesized by nucleophilic addition of hydrogen cyanide to aldehyde's or ketone's carbonyl group and application of HNLs for this process provides not only excellent enantioselectivities, but also high rates and mild reaction conditions [2].

In our research we have used hydroxynitrile lyase from *Arabidopsis thaliana* (*At*HNL), which is a first (*R*)-selective enzyme with an $\alpha/\ \beta$ hydrolase fold identified from HNLs' superfamily [3]. The enzyme was covalently immobilized on a siliceous monolithic microreactor and used in a continuous flow system for the hydrocyanation of benzaldehyde and *p*-substituted benzaldehydes. Reactions were carried out in buffer saturated MTBE pH 5.4 to suppress the non-selective reaction [4]. The studies have aimed to investigate the *At*HNL-catalyzed hydrocyanation in a continuous flow microreactor to achieve maximum control over the process in a minimum time frame.

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Keywords: chiral cyanohydrins, hydroxynitrile lyase, microreactor, flow chemistry, silica monolith

P-20: Innovative cosmetic formulations with microalgae extract – preparation and testing

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Microalgae are a great source of many valuable compounds and their extracts are an interesting addition to cosmetics, mainly due to the presence of carotenoids and vitamins from group A. Vitamin A contributes to the reconstruction of the epidermis and strengthening its protective functions. It also supports the synthesis of the basic building blocks of the skin - collagen and elastin. The number of beneficial properties can be also attributed to carotenoids, as they are the precursors of vitamin A. The most important role is the protection of skin components against free radicals resulting from damage caused by the UV radiation. Carotenoid extracts are used most often in moisturizing and regenerating cosmetics, but also in creams protecting skin and hair against the UV radiation.

The main aim of the project was concentrated on checking whether the use of the self-obtained microalgae extract can improve the usability properties of the exemplary cosmetic formulations and improve the condition of the skin. The carotenoid extracts were obtained from three species of microalgae: *Dunaliella tertiolecta*, *Cylindrotheca closterium* and *Rhodomonasmaculata*. Two cosmetic formulations of the W/O and O/W type with and without the extracts (placebo) were prepared. Products have passed the stability and preservative efficacy tests. The product's sensory properties during application and use were identified and quantified by a group of 10 volunteers. Furthermore, the hydration of the epidermis was evaluated with a corneometer, which measures the hydration level of the superficial layers of the skin (stratum corneum) based on electrical conductivity. The results obtained for the received cosmetic formulations with microalgae extracts are promising and show that the self-obtained extracts may be of beneficial properties to cosmetics.

The research was financed under the student project no. 0170/2020 by the Warsaw University of Technology from the Large Pool of Scientific Circle Council.

Keywords: microalgae, natural cosmetics, carotenoids

P-21: P-solubilizing *Streptomyces roseocinereus* MS1B15 with multiple plant growth promotion increased barley plant growth, and soil nutrients

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Phosphate-solubilizing bacteria (PSB) have been reported to increase phosphate (P) content and plant growth. Their application in agricultural systems is an eco-friendly alternative strategy for limiting negative environmental impact of chemical fertilizers and increasing costs. Therefore, the aim of this study was to isolate and characterize new putative PSB and evaluate its Plant growth promotion (PGP) attributes to be used as bio-inoculum to enhance plant growth and increase P bioavailability in soil. Sixteen bacteria were isolated from Moroccan oat rhizosphere and were screened for their putative P-solubilization by semi-quantitative agar spot method. The two strains MS1B15 and MS1B13, identified as S*treptomyces roseocinereus* and *Streptomyces natalensis*, respectively, showed the maximum phosphate solubilization index (PSI = 1.75 and PSI = 1.63). The liquid assay demonstrated that *Streptomyces roseocinereus* MS1B15 had a strong P solubilization and was selected to evaluate its putative plant growth promotion activities including production of siderophores, indole-3-acetic acid (IAA) and amino-cyclopropane-1-carboxylate (ACC) deaminase, nitrogen fixation and antimicrobial activity against soil-borne plant pathogens. Under greenhouse condition, barley plants inoculated with *S. roseocinereus* MS1B15 significantly increased shoot and ear length as well as available phosphorus in ears and leaves and P and N contents in the soil. Overall results showed that the selected strain S. roseocinereus MS1B15 could represent a potential candidate as biofertilizer to increase plant growth as well as P uptake.

Keywords: phosphate solubilizing bacteria, plant growth-promoting rhizobacteria, barley growth, P availability

P-22: Analysis of *OGG1* and *MUTYH* gene expression in two brain regions of rats subjected to chronic mild stress and during escitalopram drug intake

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Major depressive disorder (MDD) is one of the most popular mental illness of these days. There are more than 246 people affected worldwide. Despite the availability of various treatments, there is still high percentage of patients resistant to them, usually due to the lack of molecular diagnosis of disease. For this reason, there is a continuous need to extend the research on molecular mechanism of depression. There are the evidences that impairments of DNA repair mechanisms are involved in depression. Moreover, the appearance of single-nucleotide polymorphisms (SNPs) in genes encoding proteins of base excision repair (BER) was observed, which may lead to less efficient DNA repair mechanisms in patients with MDD. As a part of this research we decided to analyze expression of these genes in chronic mild stress (CMS) model of depression in rats, including the treatment with a drug escitalopram, belonging to the group of selective serotonin reuptake inhibitors (SSRI). Therefore, adult male Wistar rats were subjected to the CMS for 2 weeks. Then both control and stressed groups were further divided into matched subgroups to receive administration of placebo or escitalopram drug for the subsequent 5 weeks. After that, mRNA level of two genes, *OGG1* and *MUTYH* was examined in chosen brain tissues, midbrain and hypothalamus. Expression of these two genes was analyzed by real-time PCR. In comparison to control, stressed rats with placebo present an increase *OGG1* and *MUTYH* gene expression level in both hypothalamus and midbrain. Nevertheless, in case of drug application, expression of both genes in hypothalamus and midbrain decreased significantly. In conclusion, we can observe that expression of genes involved in BER can be modulated in occurrence of depression and its treatment with inhibitors of selective serotonin reuptake. Also, it suggests that SSRI could be a good treatment for patients with depression and BER mechanisms impairment.

Keywords: major depressive disorder, DNA repair, base excision repair, gene expression

P-23: Analysis of gene expression promoting proliferation in squamous colorectal cancer

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Currently, cancer is one of the most serious civilization diseases, and its treatment is a challenge for modern medicine. Despite accelerating progress in the development of anti-cancer therapies, there is still a dedication to developing medicines for malignant neoplasms that are very difficult or impossible to cure. One such group of neoplasms is squamous cell carcinoma. Squamous cell carcinoma of the colon is extremely poorly known. Due to the emerging reports on transmembrane transporters responsible for the transport of ions, among others, sodium and potassium, with the proliferation and progression of neoplasms, the effect of increased concentrations of these ions on survival, viability, and gene expression with proliferation and apoptosis in tumor cells of the DLD-1 line was investigated. Survival of DLD-1 cells determined by the trypan blue test. Viability was determined by the MTT test. Both investigations showed a significant effect of potassium ions on the decrease in the survival and viability of the tested cell line compared to healthy cells - fibroblasts. The qPCR analysis showed an increased expression of the KCC4 gene in tumor cells, which decreased statistically significantly due to the increased concentrations of NaCl and KCl. The observed decrease in expression is probably associated with a decrease in the viability and proliferation potential of cancer cells.

Keywords: squamous cell carcinoma, gene expression, potassium ions, sodium ions, KCC

P-24: Isolation of exosomes excreted by SW480 cancer cells and their preparation for drugs loading

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Exosomes are small in size, 30 to 100 nm in diameter, membrane bound vesicles that are naturally released from a cell. Exosome membrane contains a rich variety of bioactive molecules, including receptors, ion-channels, and molecules specifically characterizing exosomes and other biomarkers dependent on the type and state of the cell of origin. Exosomes are able to influence the homeostatic processes in recipient cells and reprogram the cell cycle, including survival, proliferation, apoptosis, differentiation, tumorigenesis, and metabolism. The cell-to-cell communication enables mediating adaptive and innate immune responses, for instance responses of dendritic cells to pathogens or cancer. The exosomal intercellular communication may increase the survival and growth of tumor tissue by releasing exosomes that enhance angiogenesis and metastasis. Because of the active participation of exosomes in the intercellular communication and cell-to-cell delivery of bioactive and genetically significant cargo, the therapeutic potential of extracellular vesicles cannot be overestimated. Since exosomes released by cancer cells trigger tumorigenic responses, hence controlling the secretion of exosomes, their content, and the internalization in recipient cells have been a major challenge for pharmacological industry. In this work, we have isolated exosomes from SW480 cancer cells using the ultracentrifugation method. The presence of exosomes was evaluated by SDS-PAGE, Western-Blotting (WB), nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). The monoclonal antibodies against CD9 protein were used. An efficient method for loading of doxorubicin anticancer drug to exosome nanocarriers has been developed. The drug content in exosomes was evaluated using fluorescence methods.

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Keywords: exosomes, drug nanocarrier, doxorubicin, ultracentrifugation, fluorescence

P-25: Evaluation of platinum nanoparticles-doxorubicin interactions

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For many years, nanotechnology has been used in many fields of science. Scientists are trying to find new applications of nanomaterials in medicine, as they seem to be great candidates for revolutionizing the methods of diagnosis and treatment of human diseases [1].

One approach is to obtain efficient nanocarriers for drugs, for example cancer therapeutics. Such carriers may be helpful in improving the targeting of drugs to specific tissues, thereby reducing their aggressiveness towards healthy cells, and increasing the anti-tumor activity of both agents. However, the first step in such research should be to test the interactions between a potential nanocarrier and the target drug, as well as its ability to modulate the biological activity of the drug [1,2].

Here, we focus on study of interactions between platinum nanoparticles (PtNPs) and doxorubicin, which is commonly used therapeutic in cancer chemotherapy [3]. Our studies are conducted to demonstrate the potential interactions between 50 nm platinum nanoparticles and doxorubicin using biophysical methods, including fluorescence spectroscopy, dynamic light scattering as well as Isothermal titration calorimetry. The results obtained so far indicate potential interactions between DOX-PtNPs, however the aim is also to check mutagenic and cytotoxic activity of these compounds.

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Keywords: nanoparticles, platinum, doxorubicin, drug delivery system

P-26: Production and characterization of monoclonal antibodies specific to different isoforms of bacteriocin BacSp222 using hybridoma technology

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BacSp222 is a recently discovered peptide bacteriocin produced by *Staphylococcus pseudintermedius* strain 222. It has a cationic charge and consists of 50 amino acids forming four alpha-helices. We can distinguish three isoforms of bacteriocin, one without posttranslational modification and two isoforms with succinyl groups on lysine residues.

In micromol concentrations, BacSp222 kills a broad spectrum of Gram-positive bacteria, but interestingly, it is also cytotoxic toward eukaryotic cells. In contrast, at nanomolar concentrations the bacteriocin demonstrates a strong proinflammatory and immunomodulatory effect toward murine macrophage cells, manifesting in increased expression of inducible nitric oxide synthase. The dual mechanism of action of bacteriocin on eukaryotic cells is unknown and requires further study.

For this purpose, our team decided to produce monoclonal antibodies specific to two isoforms of the bacteriocin BacSp222 using hybridoma technology. The first step was the immunization of BALB/c strain mice with the appropriate isoform of bacteriocin. After killing the mice, spleens were isolated from them, and splenocytes were immortalized by fusion with SP2/0-Ag14 mouse myeloma cells using polyethylene glycol. We obtained six hybridoma cell lines producing monoclonal antibodies specific to bacteriocin. Isotyping test revealed that all selected antibodies were IgMs class. Monoclonal antibodies were purified from the cultured media by affinity chromatography on either protein L or CaptureSelect™ IgM affinity matrix. One of the antibodies was biotinylated to test the possibility of using a sandwich ELISA. The produced monoclonal antibodies will serve as tools in immunofluorescence and flow cytometry, allowing us to understand the mechanism of bacteriocin interactions with eukaryotic cells.

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Keywords: bacteriocin, BacSp222, monoclonal antibodies, hybridoma technology

P-27: Does light exposure trigger pheomelanin production in fungi? – EPR studies

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Light plays a crucial role in the development of many living organisms. It may affects also on fungi growth, their life strategies [1] or pigments production [2]. One of the most ubiquitous fungal pigments are melanins, which are postulated to play a role in thermoregulation, protection against oxidative stress and radiation damages [3]. There are many publications considering function of eumelanins and 1,8-dihydroxynaphtalene melanins [3], but the role of fungal pheomelanins is poorly understood. Here we present how white light exposure, in comparison to cultivation in the dark, impacts pheomelanin production in *Leptosphaeria biglobosa* F80 (Kowalski), the well known pathogen of oilseed rape and potential endophyte of *Fraxinus excelsior* L. [4]. Fungal colonies were cultivated in the malt extract (ME) medium (Biocorp). Homogenised mycelia were analysed on magnettech miniscope MS 300 EPR spectrometer at the temperature of liquid nitrogen (77 K), with the following parameters: B0 3365 G, modulation amplitude 2 G, sweep time 180 s, accumulations 3. Phototropism experiments were conducted using plate ME cultures. Our study reveals how light exposure affects pheomelanin synthesis qualitatively and overall melanin quantitatively.

Additionally, the tropism of the fungal growth and melanin synthesis toward light source were also analysed. The studies shed a light on how this ecologically important pheomelanin-producing fungi reacts on white light, and what is the role of pheomelanin pigment in fungal reaction to light stress.

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Keywords: pheomelanin, light, *Leptosphaeria biglobosa*, EPR

P-28: Sewage as a rich source for isolation of host-specific bacteriophages against bacteria from *Enterobacteriaceae* family

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Bacteriophages are viruses capable of infecting and replicating inside bacteria or archaea cells. When virulent – they lead to the destruction of cells by lysis. Therefore, they are an effective weapon against bacterial pathogens. Their use in therapy is considered safe, as they do not infect animal and human somatic cells. They also have the potential to eradicate the so-called "super bacteria" – antibiotic- and multidrug-resistant bacteria.

There are many sources for bacteriophage isolation, especially those high in the number of various bacterial strains like sewage, cesspits, slurry, water, or soil. This study aimed to evaluate if petroleum-derived sewage coming from a wastewater treatment plant could be a source for lytic, Enterobacteriaceae infecting, phages isolation. In this work, phages were successfully isolated. After multi-step sample preparation and purification, a spot-test was made. As a result, bacteriophages able to infect bacterial strains from the Enterobacteriaceae family –*S. typhimurium*, *S. enteritidis*, and *E. coli* were found. These pathogens often cause diseases of the digestive system, including poisoning. Moreover, they lead to food industry problems - as they can be spotted in water and comestible products, such as meat, eggs, and dairy.

In the USA it is possible to use bacteriophage-based products, being phage cocktails, for combating bacterial food contamination. For example, Listex™ - food disinfectant eliminating bacteria from the Listeria genus. Then, against the Salmonella genus, there is a German product, SalmoFresh™. As it can be seen, bacteriophages slowly step in the food protection and disinfection industry, and their potential allows them to be used in even more industries. Further research on isolated bacteriophages may lead to formulating and later applying such cocktails for food protection or water purification, and therefore expanding the use of phages as germicides in Poland.

Keywords: bacteriophage, Enterobacteriaceae, isolation

P-29: Develop new inhibitors of II type secretion system from *Pseudomonas aeroginosa*

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Pseudomonas aeroginosa belongs to the gram-positive opportunistic bacteria that induce infections in humans. These microorganisms possess the ability to form cell biofilms and are resistant to widely used antibiotics, which affects the high virulence of this species. *P. aeroginosa* are one of the main causes of nosocomial infections among people with reduced immunity for example with diseases such as cystic fibrosis, cancer and diabetes.

The secretion system of this pathogen is mainly based on three virulence systems, of which type 2 secretion (T2SS) and type 3 (T3SS) are responsible for the secretion of effector proteins that perform essential function in the pathogenesis process. For example, the type 2 secretion system is responsible for the secretion of exotoxin A (ExoA) causing inhibition of protein synthesis in host cells, thus leads to their damage. The strategy to bypass antibiotic resistance is to inhibit or impair major secretion systems that are responsible for high virulence and the ability to inactivate antibiotics in *Pseudomonas aeroginosa.*

The aim of the project was to develop type II secretion inhibitors (specific virulence blockers) supported by molecular modeling (in-slico-optimized) for the *Pseudomonas aeruginosa* pathogen.

Research was founded by National Science Center (2016/21/BNZ6/02028) and the Ministry of Science and Higher Education (6878/II-LAN/SP/2018).

Keywords: inhibitors, *Pseudomonas aeroginosa*, II type secretion, molecular modeling, virulence blockers

P-30: Analysis of copy number of selected chromosomes in hybrid embryos (*Bos taurus* × *Felis catus*)

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Hybrids are a valuable model used for basic research on the mechanisms of fertility disorders, embryology or cytogenetics, as well as research on the conservation of species threatened with extinction. Combining gametes from two different species is also used to assess the fertilization ability of semen and the interaction of spermatozoa with the oocyte in different species. The availability of gametes from wild felids is very limited, therefore their oocytes are particularly valuable. Domestic cat is frequently used in research as a model for endangered species of the *felidae* family. The aim of this study was to analyze copy number of selected chromosomes in hybrid embryos obtained after *in vitro* fertilization (IVF) using fluorescent *in situ* hybridization (FISH) method.

Total of 177 cumulus-oocytes complexes (COCs) were collected from 18 domestic cat ovaries after ovariohysterectomy. Oocytes were matured *in vitro* and fertilized using bovine semen. Presumptive zygotes were cultured *in vitro* for 30 hours and fixed on glass slides. FISH with three painting probes specific for X and Y bovine chromosome and X feline chromosome was performed on them. Slides were evaluated using a fluorescence microscope and Zeiss ZEN imager software.

As a result of IVF, 4 two-blastomere interspecies embryos were obtained. Presence of molecular probes complementary to chromosomes of domestic cat and domestic cattle was observed. Uncleaved embryos during nucleus division and two-blastomere embryos during cytoplasm division were also identified.

In conclusion, the study indicates a possibility of creating taxonomically distant hybrids at the embryonic level and analysis of slides containing interphase nuclei. Using substitutive gametes offers the opportunity to develop new and improve existing methods of assisted reproduction techniques and cytogenetics through the use of substitutive gametes.

Keywords: hybrids, *in vitro* fertilization, fluorescent *in situ* hybridization, embryology, cytogenetics

P-31: Pipeline for diversity assessment of eukaryotic rhodopsins in metatranscriptomic data

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Rhodopsins are photosensitive transmembrane proteins performing various functions, such as ion pumping and light sensing. Some are also responsible for light-induced energy generation – an alternative process to photosynthesis. The main goal of this study was to develop a pipeline for eukaryotic rhodopsins' analysis and assess their diversity in published data and, ultimately, also for the data from Lake Roś generated in our laboratory. The standalone pipeline for basic data analysis and rhodopsin studies was prepared and tested. The pipeline includes quality check (FastQC), mRNA and rRNA sorting (SortMeRNA), taxonomic assignment using both mRNA (TaxMapper and BLAST) and rRNA (phyloFlash) fraction, and functional annotation of transcribed genes (TransDecoder and eggNOG). The general performance of the pipeline was validated on two metatranscriptomic datasets from freshwater environments and rabbit cecal content. For rhodopsin searches, graftM, MMseqs2 and MetaEuk tools were tested on two transcriptomes from the genus *Chlamydomonas*, a metatranscriptome from Arctic fiords, and a metagenome from Lake Mendota. The analysis of most of the data sets shows that all three tools provide comparable results.However, the MMseqs2 seems to be the most sensitive, and only MetaEuk enables the identification of viral rhodopsins. To check if any groups of rhodopsins are overrepresented in the data, a cluster analysis was performed. The results suggest that some sequences could be classified as halorhodopsins, sensory rhodopsins type III or cruxrhodopsins only by MMseqs2. Moreover, viral rhodopsins were misclassified by all tools except MetaEuk. To increase the sensitivity of the pipeline, eukaryotic sequences were identified in publicly available eukaryotic genomes and added to the rhodopsin database (MicRhoDE). A new curated database was tested on the same datasets and resulted in identification of 35828 new rhodopsins in metatranscriptomes and 264763 in metagenomes.

Keywords: metatranscriptomics, metagenomics, rhodopsin, freshwater, Eukaryota

P-32: Western diet (WD) may trigger brain changes typical for Alzheimer's-like neurodegeneration

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The Western diet (WD) is a type of nourishment characterized by a large amount of highly processed food, rich in simple sugars and saturated fats and, with high value of the insulin and glycemic index. Long-term consumption of WD leads to metabolic syndrome including chronic hyperglycemia, hyperinsulinemia, hypercholesterolemia, insulin resistance and liver dysfunction. The metabolic disorders are described as probable risk factors inducing neurodegenerative processes related to tau protein pathology, observed *e.g.* in Alzheimer's disease. Clinically, AD is characterized by impairment in cognitive functions, and neuropathologically by the deposition of amyloid-β plaques and neurofibrillary tangles composed of phosphorylated tau protein. The propagation of AD pathology in the brain starts from temporal area including entorhinal cortex and the hippocampus responsible for memory processes and cognition.

The aim of the study was to determine whether WD-derived metabolic disorders may impair cerebral insulin signaling and whether such disturbances may induce tau protein pathology in the brain.

An experiment was performed in males of wild type C57BL/6 mice fed with WD or standard diet (SD). Because the neurodegenerative changes are age-dependent, the experimental groups (WD and CTR) of mice were divided into three age subgroups 4-, 8-, and 12-month-old. In the first part of the experiment we analyzed the development of peripheral metabolic changes induced by WD. In the second part, we assessed whether peripheral disorders may change the levels of insulin resistance marker - pIRS(Ser616) and pTau(Thr231) in the brain.

The Western blot analysis of mice brain lysates revealed an increase in the level of the pIRS (Ser616) in the entorhinal cortex area, what suggests that WD-related brain insulin resistance may be linked to cognitive impairment. Obtained results suggest that western diet may be consider as an important modifiable risk factor of neurodegenerative diseases.

Keywords: Western diet, metabolic syndrome, insulin resistance, neurodegenerative diseases

P-33: The newly discovered, ammonium-tolerant microalga *Chlorella vulgaris* K-01 and its superpowers

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Cell walls of *Chlorococcales* green algae are complex polymeric structures consisting of cellulose, hemicelluloses, pectins, proteins and, in many cases, a hydrocarbonaceous polymer – algaenan. The presence of algaenan in the outer layer results in cell wall resistance to treatment with many lytic enzymes and non-oxidizing chemicals. Algaenan severely limits and sometimes completely prevents the extraction of biotechnologically useful metabolites from the algal biomass. Therefore, the study of the structure of cell walls and the effects of abiotic environmental factors on their biochemical composition are crucial for the development of future biotechnologies based on microalgae.

In the present study, we have evaluated viability and metabolic activity of the microalgal strain K-01, isolated initially from the efflux of the brine graduation tower (Ciechocinek, Poland) and identified as *Chlorella vulgaris*, grown in axenic cultures.

The examined strain expressed an extremely high tolerance to ammonium ions. Photosystem II activity as measured by chlorophyll fluorescence induction was not affected up to the concentration of 112 mM ammonium chloride in the medium, which is nearly three times more, than reported earlier for *Chlorococcales*. The supplementation of culture media with carbohydrates (glucose, sucrose) resulted in a switch to the heterotrophic growth in the presence of high ammonium concentrations. The cell walls purified from K-01 growing on ammonium-rich media presented different protein composition (SDS-PAGE), suggesting an effect of elevated ammonium concentrations on cell wall structure. These properties show a substantial potential of *Chlorella vulgaris* K-01 for the treatment of eutrophicated waters and the use of such waters to obtain algal biomass applicable for further processing.

Keywords: *Chlorella vulgaris*, *Scenedesmus obliquus*, cell walls, ammonium ions, algaenan

P-34: Altered metabolism of auxin in *Arabidopsis thaliana* under ammonium nutrition

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Plants can use ammonium and nitrate ions as a source of inorganic nitrogen, but when ammonium ions serve as the only source of nitrogen, symptoms of ammonium syndrome are observed. In *Arabidopsis thaliana* it is synonymous with alterations in intracellular metabolic level, but it also radically stops the growth of the plants. Hormones are substances involved in the regulation of plant development, of which mostly auxin can regulate the growth of plant organs. Auxin is synthesized mainly in stem apex and are transferred through the whole plant. Auxin can create conjugates with amino acids, glucose and be oxidized which can cause its degradation or inactivation. The study aimed to test whether changes in auxin metabolism are responsible for Arabidopsis phenotype under ammonium nutrition.

It was observed that ammonium syndrome causes higher concentration of auxin both in leaves and roots. It also alters transcript level of genes involved in conjugation of auxin as well as the concentration of derivatives. Arabidopsis GUS and GFP lines helped visualize changes in local auxin gradients in tissues in ammonium nutrition. It can be concluded that ammonium nutrition changes pathways of auxin metabolism on many levels such as synthesis, conjugation and degradation.

Founded by Polish National Science Centre, grant no. 2018/29/B/NZ3/02687.

Keywords: auxin, *Arabidopsis thaliana*, ammonium nutrition

P-35: High concentrations of saturated and polyunsaturated fatty acids regulate the activity of autophagy in hypertrophic adipocytes

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Autophagy is a lysosome-dependent intracellular process regulating the turnover of cellular proteins and organelles. Studies have shown that autophagy may be altered in adipose tissue of obese individuals. Hypertrophic adipocytes undergo endoplasmic reticulum stress induced by obesity, which leads to adipocyte dysfunction and metabolic disorders. Decreased autophagy may slow down the removal of dysfunctional/damaged molecules and organelles, the accumulation of which contributes to cellular stresses and leads to insulin resistance. Due to the fact that high fat diet is one of the main factors inducing obesity, and different fatty acids exert different effects on metabolic processes in adipose tissue, the aim of the present study was to examine the role of saturated and unsaturated fatty acids (palmitic – PA, docosahexaenoic – DHA and eicosapentaenoic -– EPA) in regulation of autophagy in hypertrophic 3T3-L1 murine adipocytes. 3T3-L1 cells were subjected to *in vitro* adipogenic differentiation for 6 day, following hypertrophy induction by supplementation of the culture medium with 0.5 mM PA for 6 subsequent days. On the 12th day post differentiation cells were treated for 24h with experimental medium containing 0.5 mM PA, 0.1 mM EPA or 0.1 mM DHA (control cells were cultured in non-supplemented medium). Oil red O staining allowed assessing lipid accumulation in adipocytes. Western blot was used to analyzed the level of autophagic proteins: LC3, beclin 1, Atg5. Oil red O staining confirmed hypertrophy induction in cells cultured for 6 days in medium supplemented with 0.5 mM PA. Western-blot analysis showed significantly decreased levels of autophagic markers: LC3-II and Atg5 in hypertrophic fat cells, with the lowest levels of LC3-II in cells treated with DHA. Results obtained demonstrated that high concentrations of fatty acids induced hypertrophy, which is linked with attenuation of autophagic activity. Regulation of autophagy depends on the type of fatty acids.

Keywords: adipocyte, autophagy, fatty acids

P-36: Electrochemically controlled modification of polyamide with nanoparticles of antibacterial properties

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Electrochemically controlled nylon-6.6 synthesis and modification was performed at the polarized liquid – liquid interface. Nylon® is a popular and interesting polyamide belonging to the family of polymers. It has high mechanical strength and has a wide range of applications in many industrial sectors. Nylon synthesis can be performed in a spectacular way known as the nylon rope trick (click to see a video). We have found that this reaction can be controlled at ITIES [1].

Nylon synthesis and modification was performed using the liquid–liquid interfaces known in electrochemistry as ITIES – Interface Between Two Immiscible Electrolyte Solutions. It allows the study of the interfacial charge transfer under the influence of the applied voltage. Controlling or following this phenomenon can be achieved with e.g. Ion Transfer Voltammetry (ITV), which measures the ionic current under the influence of an applied interfacial potential difference.

In this work, we have focused on the interfacial modification of Nylon® with silver nanoparticles. Ag^+ ions initially present in the aqueous phase was reduced to metallic silver by ferrocene from the organic phase. Parameters such as the effect of the potential sweep rate, the effect of the concentration of diamine in the water phase and adipoyl chloride in the organic phase on the interfacial polymerization and doping were investigated and optimized. The influence of silver nitrate concentration initially dissolved in the water phase on the electrochemical signal was investigated. The influence of ferrocene on the formation of nylon alone and nylon doped with Ag NPs at ITIES was also investigated. The material formed at the LLI was additionally characterized with infrared (IR) spectroscopy and scanning electron microscopy (SEM).

The research was financed by the NCN Poland as part of the PRELUDIUM 19 project (UMO-2020/37/N/ST4/00270).

[1] K. Kowalewska, K. Sipa, A. Leniart, S. Skrzypek, L. Poltorak, *Electrochemistry Communications*, 2020, 115, 106732.

Keywords: polarized liquid–liquid interface, ITIES, polyamide film, interfacial modification

P-37: Miniaturization of polarized liquid-liquid interface as a powerful tool in quinine determination in real samples

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Quinine (QN) is a natural alkaloid, extracted for the first time from the bark of the South American cinchona tree. QN is non-volatile, crystalline solid. It is the oldest antimalarial agent. Despite a relatively high toxicity QN is also used in tonic water as a flavor component. The maximum allowed content is 7.5 ml of QN hydrochloride per 100 ml of drink [1].

In recent years, we increasingly hear about cheap tricks of producers, mainly in the food and pharmaceutical industries, e.g they present false product compositions. Hence, at least one is removed from the market every day. The raising consciousness related to "green chemistry" stimulate researcher to start looking for a new, cheap and sensitive method allowing the determination of specific substances. Our method relays on electrochemistry at the Interface Between Two Immiscible Electrolyte Solutions (ITIES) in conjunction with Ion Transfer Voltammetry (ITV). The applicability of the developed method was achieved via liquid-liquid interface miniaturization. In this respect, the specially designed voltammetric cells with fused silica microcapillary having 25 µm as an inner diameter was used [2]. Tonic samples were purchase form three independent producers. We have found that the µITIES is a powerful tool in determination of QN in real samples.

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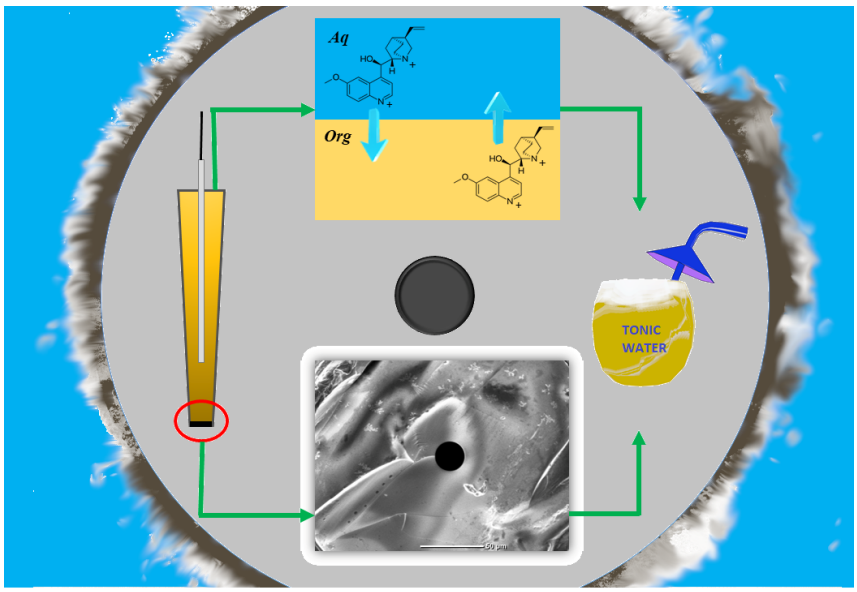
[1] Z. Koczorowski, Z. Figaszewski, A. D. Petelska, *Elektrochemia Cieczowych Granic Fazowych*, Wydawnictwo Uniwersytetu Warszawskiego, Warszawa, 2011
[2] Polish Patent Application no. P.436383

Keywords: quinine, mikro-ITIES, tonic water, electrochemistry



P-38: Expression of *IKBKB* in peripheral blood and brain regions of rats subjected to chronic mild stress and venlafaxine treatment

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Major depressive disorder (MDD) is a significant contributor to the global burden of disease and affects people across the world. Due to the constantly increasing number of patients, it is estimated to be the second leading cause of social disability. Despite the importance of the problem, pathogenesis of the disease is not fully understood. Nevertheless, growing amount of evidence suggest that MDD could be elicited by the elevated activity of proinflammatory molecules, both at the periphery and within central nervous system. One of the inflammatory systems dysregulated in MDD is NF-kB pathway. Normal NF-kB activity is also essential for brain functioning and neuronal plasticity. Canonical signaling of NF-kB is activated by the IkB kinase, consisting of three subunits, one of which is IKK-B encoded by *IKBKB* gene. Accordingly, alterations in *IKBKB* gene expression can disrupt the NF-kB system and may influence the development of MDD. Therefore, the present study investigates whether: (1) the CMS procedure, which closely mirrors depression in humans induce changes in IKBKB expression in peripheral blood mononuclear cells (PBMCs) and in selected brain structures; (2) chronic administration of serotonin-norepinephrine reuptake inhibitor, venlafaxine, alters the expression of *IKBKB* gene.

Adult male Wistar rats were subjected to the CMS procedure. After 2 weeks, both control and stress groups were further divided into subgroups to receive administration of vehicle or venlafaxine for the subsequent 5 weeks. Real-time PCR was used to analyze the mRNA level of *IKBKB*. The results show higher expression of *IKBKB* in the blood of CMS rats and decrease of *IKBKB* level after venlafaxine treatment. Simultaneously, expression of *IKBKB* in the hippocampus, amygdala and midbrain of was significantly diminished after stress procedure. This data suggest that *IKBKB* gene could be implicated in the activation of inflammatory pathways in MDD.

Keywords: depression, inflammation, chronic mild stress

P-39: *In vivo* analysis of antimicrobial activity of phages infecting *Pseudomonas* spp., *Serratia* spp. and *Aeromonas* spp.

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In the era of increasing antibiotic resistance of bacteria, bacteriophages are consider to be effective treatment factor of bacterial infections.

The aim of the project was to evaluate the antibacterial properties of phages infecting opportunistic pathogens of humans: *Pseudomonas* spp., *Serratia* spp. and *Aeromonas* spp. Phages used in test were isolated from Wołomin sewage treatment plant: WOL2, STU26 and PRA26 - phages of *Pseudomonas* and from a mine in Złoty Stok: *Serratia* phage BZS1 and *Aeromonas* phages MZS3, MZS4.

To test the efficacy of phage therapy we used method based on the survival of honeycomb moth larvae (*Galleria mellonella*). These larvae were injected with a lethal dose of bacteria (causing death of 50% of the larvae after 24 hours) and then with a phage potentially inhibiting bacterial growth. Inhibition of bacterial growth delays larval death.

First, lethal dose of bacteria was established. In the next steps we determined the amount of phages needed to inhibit bacterial growth inside the infected larva. We analysed various phage concentrations: MOI = 10, 1, 0.1 (phage particles per bacterial cell). Additionally, to test whether the time of phage injection affects the therapy efficacy, we compared various time points of phage administration: immediately and 120 minutes after injection of bacteria.

All phages showed *in vivo* antimicrobial activity against their hosts. Phage injection increased survivability of the larvae compared to control group. The obtained results showed that earlier phage administration increases the efficacy of therapy. Of all tested *Pseudomonas* phages, the most effective in delaying larvae death was phage STU26. Among phages infecting *Aeromonas* spp., MZS4 has proved to be more effective. A phage that infects bacteria of the *Serratia* genus has slight effect on inhibiting bacterial growth inside larvae.

Keywords: phages, antimicrobial activity, *Galleria mellonella*

P-40: The application of MALDI MSI technique in the evaluation of symbiosis and pathogenesis in plants

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Matrix Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI MSI) is an efficient technique used for both chemical analysis of metabolites as well as the visualization of their distribution in plant tissue. It is used to analyse biomolecules, such as lipids, sugars, proteins and small molecules, e.g. endogenous metabolites, without labelling. The use of sensitive MALDI MSI technique enables to perform the analysis *in situ* without the disruption of the tissue.

Rhizobia, are Gram-negative soil bacteria, able to enter into symbiosis with legume plants and performing the biological nitrogen fixation, in which an atmospheric nitrogen is reduced into its assimilable form such as glutamine. Many signalling compounds are involved in this process, e.g.: plant hormones, auxins, which stimulate the formation of root nodules under the influence of Nod factors.

Agrobacterium tumefaciens is also a Gram-negative soil bacterium, taxonomically belonging to the rhizobia group, but it is a phytopathogen that attack the stems and roots of many varieties of dicotyledons and some gymnosperms. *Agrobacterium* mainly attacks injured tissues, recognising them by compounds such as phenols and sugars, released at the places of injury, which act as chemoattractants. The pathogen transforms plant cells, forcing their continuous division, which leads to the formation of tumour tissue.

In our study the MALDI MSI technique was used to analyse the plant tissues under the influence of stress of bacterial infection, both symbiotic and pathogenic. Moreover, the use of spectral analyses allowed to examine significance of selected signalling compounds both in the process of biological nitrogen fixation and plants antimicrobial reaction.

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Keywords: MALDI MSI, *Rhizobium*, symbiosis, *Agrobacterium*, tumor tissue

P-41: The effect of polystyrene nanoparticles of different diameters on DNA damage in peripheral blood mononuclear cells

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In recent decades, the production and use of plastics has greatly increased. Plastics can be fragmented into microparticles with a diameter < 5000 nm, and then to nanoparticles with a diameter < 100 nm. Plastic NPs may enter living organisms from air, food, and water, and also through skin. Then, they accumulate in the subsequent links of the food chain.

One of the main plastics used for packaging, commercial, and construction purposes is polystyrene (PS). It is a petroleum-based material obtained by polymerization of styrene (vinylbenzene) monomers. Thus far, the carcinogenic effect of styrene has been confirmed, however, there are no reports indicating the carcinogenic effect of its derivative — polystyrene. Nonetheless, the latest data suggest that polystyrene nanoparticles (PS-NPs) can induce oxidative stress, contributing to tissue inflammation and necrosis.

NP-PSs can penetrate the circulatory system, which is why it is important to assess their genotoxic properties in peripheral blood mononuclear cells (PBMCs). Genotoxic damage of PBMCs can contribute to immune system disorders, which can lead to the development of cancer or autoimmune diseases (e.g. asthma, allergies).

The aim of our study was to evaluate the effect of PS-NPs of various diameters (29, 44 and 72 nm) on DNA damage in human peripheral blood mononuclear cells. This analysis was performed using the comet test (single-cell gel electrophoresis). Cells were incubated with PS-NPs in the concentration range of 0,0001–100 µg/ml for 24 h, and then the level of DNA damage was assessed.

The tested nanoparticles were found to increase DNA damage. The largest changes of the examined parameter are caused by nanoparticles with a diameter of 29 nm, while the smallest changes are caused by nanoparticles with a diameter of 72 nm.

Keywords: polystyrene nanoparticles, PBMCs, DNA damage, genotoxicity

P-42: Cyclosporine A changes expression of TGFβ1-3 in keratinocytes treated with LPS

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Background: Cyclosporine A (CsA) coventional therapy is used in the treatment of moderate to severe psoriasis.

Aim: The purpose of this study was to evaluate changes in the expression of transforming growth factor β 1-3 (TGFβ1-3) in human keratinocyte (HaCaT) culture exposed to lipopolysaccharide A (LPS).

Material and methods: HaCaT culture was exposed to 1 ng/ml LPS for 8 hours and next 100 ng/ml CsA was added for 2, 8 and 24 hours and compared to the control culture. Microarray, RTqPCR and ELISA assay were used.

Results: It was observed changes in expression of TGFβ1-3 as follows: (*TGFβ1*: H-2 vs C +8.66; H-8 vs C -2.33; H-24 vs C -1.33; *TGFβ2*: H-2 vs C +1.01; H-8 vs C -3.66; H-24 vs C -4.06;*TGFβ3*: H-2 vs C +2.22; H-8 vs C +2.18; H-24 vs C +3.01).

Conclusion: Cyclosporine A has a potential to induce changes in a transcriptional activity of genes associated with TGF β singaling pathways.

Keywords: cyclosporine A, keratinocyte, TGFβ

P-43: Effect of the rotating magnetic field on bacterial biofilm extracellular matrix physical and chemical properties

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The application of various magnetic fields (MFs) in combination with antimicrobial agents has been studied for long time and suggested as promising and effective adjunct therapy. However, this approach is still not widely accepted, from the reason of lack of knowledge concerning the possible mechanism of this phenomenon. Nowadays, there are a few assumptions which try to theoretically explain the mechanisms by which MFs can enhance effectiveness of antimicrobials. One of them concerns the influence of MFs on the physical and chemical properties of biofilm matrix.

This study aimed to analyze the effect of the rotating magnetic field (RMF) exposure on the physical and chemical properties of biofilms formed by various *Pseudomonas aeruginosa* and *Staphylococcus aureu*s strains.

The exposure of purified from bacterial cells biofilm matrix to RMF was carried out using a self-designed set-up containing a generator of RMF, made of a three-phase stator of an induction squirrel cage motor. After exposition, the purified biofilm matrix composition was analyzed by NMR, FTIR, LC-MS/MS and GC-MS methods.

The obtained results showed that, RMF modified a number of biofilm matrix physical and chemical properties. The effects were depended on magnetic flux characteristic, time of exposition, as well as specific strain of bacterial species. Results of performed studies shed a new light into our understanding of biofilm tolerance and resistance on electromagnetic filed influence and may open up new possibilities for ways of biofilm's eradication.

The work was supported by the National Science Centre, Poland (grant no. 2017/27/B/NZ6/02103).

Keywords: RMF, biofilm eradication, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

P-44: 18S rDNA amplicon-based analysis of the diversity of microbial eukaryotes communities in the salinity gradient of Świna river estuary

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The Baltic Sea is under the impact of rivers and limited inflow of high-saline waters from the North Sea. It creates an environment with a salinity gradient that is more brackish than marine. Such an environment can be home to unique communities of eukaryotic microorganisms, which play essential roles on different trophic levels. This study aimed to compare the diversity of eukaryotic microorganisms along the salinity gradient of Świna river in north-western Poland. Samples were collected in August 2018 from five stations, located in the river connected with Świna and Szczecin Lagoon, marine waters, and waters located near Świnoujście LNG terminal. Physicochemical data were also measured. To analyze diversity in the environmental samples, the hypervariable region V9 of 18S rDNA gene – a part of eukaryotic small ribosomal subunit – was amplified and sequenced on Illumina platform. Sequences were analyzed by the DADA2 package in the QIIME2 environment. As a result, we obtained 3 211 ASVs (*amplicon sequencing variant*) representing different organisms. The taxonomic classification was assigned using SILVA database, and for ciliates, we also tested the classification method based on the reference tree. We estimated alpha and beta diversity for all sampling spots. Identified organisms belonged to ten main taxonomic groups: SAR, *Archaeplastida*, *Discoba*, *Opisthokonta*, *Cryptophyceae*, *Picozoa* and *Amoebozoa*. Stramenopiles, such as *Ochrophyta*, dominated in every sample. Although there was not much difference between stations, it was observed that the river station was the most diverse one. Physicochemical data of the station near the LNG terminal were the most distinct from others, and the only one indicative of an anoxic environment. That was reflected in the high abundance of certain groups, such as *Chrysophyceae*. The classification methodology based on the reference tree, although more laborious, proved to be a more precise method of taxonomic assignment.

Keywords: 18S rDNA amplicon sequencing, eukaryotic microorganisms, protists, diversity, brackish waters

P-45: Structure-function analysis of anti-viral protein IFITM3

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Despite rapid development in antiviral research, viral infections remain a threat to humankind and a global cause of death. Novel approaches for prevention and treatment of viral diseases require extensive understanding of mechanisms of infection and host immune responses. Vertebrates' cells have evolved to defend from viral infections by various pathways of innate and adaptive immune responses. IFITM (interferon-induced transmembrane) proteins are expressed during viral infection as effectors of innate response. Their expression is stimulated by interferon – signaling protein triggering a cellular "anti-viral state". IFITM1, IFITM2 and IFITM3 proteins inhibit viral infection mainly by blocking virus entry into the cell. IFITM3 has been proved to inhibit numerous viruses e.g influenza A virus, Ebola virus and severe acute respiratory syndrome-related coronavirus. We showed that IFITM3 was able to inhibit efficiently tick-borne encephalitis virus (TBEV). To analyze the structure-function relationship in IFITM3 protein, we constructed a panel of cell lines stably producing different mutated versions of IFITM3 protein, including mutations of several posttranslational modification sites. A549 cells were transduced with retroviral vectors carrying mutated IFITM3 genes to obtain stable expression of mutated proteins. Obtained cells were tested in TBEV infectivity assay. In conclusion, we show the importance of particular IFITM3 regions and posttranslational modification sites in IFITM3 protein antiviral functions.

Keywords: IFITM3, TBEV, restriction factor, antiviral activity

P-46: Isolation and identification of microorganisms able to degrade the xenobiotics

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In the last decade the amount of plastic waste became such an important issue, that scientists from all around the world have proposed several methods for decreasing non-biodegradable plastic production or its degradation in the natural environment. Recently, it has been showed, that microorganisms could be able to degrade non-degradable polymers (such as poly(ethylene terephthalate) (PET)) thanks to the enzymes like esterases. In fact, microorganisms can use the polymer as the carbon source and cut its ester bonds. In this thesis, the xenobiotics (compounds that don't exist in natural environment and are unlikely to degrade) have been analyzed with the microorganisms isolated from the polluted environment. The enzymatic activity of microorganisms was tested and later the cultures with xenobiotics (PET and catechol) were carried out. The cultivation with PET (cut to film pieces) as the only carbon source was conducted in minimal liquid medium and the cultivation with catechol on solid medium with nutrient agar and Sabouraud respectively for bacteria and fungi was performed. Cultivation with liquid medium was conducted for 28 days and during the incubation time the culture samples were analyzed using Fenton's reaction. For solid medium the results were noticed visually. As the measurement of the fluorescence showed, the signal was different for samples from 1, 2, 3 and 4 week. This can be the proof that the PET structure was changed during the cultivation, so its polymers could be metabolized by microorganisms. For solid cultivation the change in culture's color was observed, but for now it cannot be a proof for further conclusions. The microorganisms with the most changed fluorescence signal were identified and it was noted that mostly they were *Pseudomonas* sp. and *Bacillus* sp. In the future, other analysis might be helpful, to see how the PET's structure was modified after the incubation with microorganisms.

Keywords: poly(ethylene terephthalate), biodegradation, microorganisms

P-47: Impact of chronic myeloid leukemia environment on the level of DNA-double stand break and activation of repair pathways

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Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder driven by oncoprotein BCR-ABL1. The activity of pro-survival pathways and loss of genome stability in leukemic cells play a major role in leukemia progression and development of therapy resistance. Accumulation of DNA double-stand breaks (DSB) leads to mutations in DNA, what contributes to clonal evolution of cancer. In eukaryotic cells there are to two major pathways for repairing DSB: homologous recombination (HR) and non-homologous DNA end joining (NHEJ). Leukemia cells during migration change environment from high oxygen in blood circulation to hypoxia in the bone marrow (BM). Leukemic cells when residue in the BM interact with other cellular components of the BM niche. Our aim was to study how oxygen concentration and interaction with human bone marrow stroma fibroblasts influence DSB repair pathways in CML.

The study was performed on K-562 cells – a human CML model cell line. The cells were cultivated under normoxia (atmospheric O_2, 5% CO_2) in mono-culture or in the hypoxia workstation (1.5% O_2, 5% CO_2) in mono-culture or in co-culture with HS-5 cells – a model cell line of human BM fibroblasts. Using HR or NHEJ reporter plasmids and flow cytometry we analyzed activation of DSB repair pathways in CML cells. We observed that activity of HR and NHEJ pathways is affected by the conditions in which cells were cultured. This is accompanied by different level of γH2AX (a hallmark of DSB) and 8-OXOdG (a hallmark of oxidation induced damage) detected by immunostaining analyzed using flow cytometry and fluorescence microscope. Our data show that the level of DNA damage and activity of repair pathways depends on the leukemia cells microenvironment.

This work was supported by research grant from the National Science Centre UMO-2018/30/M/ /NZ3/00274 to P.P.-B.

Keywords: chronic myeloid leukemia, CML, DNA-double stand break repair, microenvironment

P-48: Selection of small molecule DDB1 ligands

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The aim of the research was to apply the molecular docking technique to identify the new small molecule DDB1 ligands. The DDB1 protein is a component of Cul4A-RING ubiquitin E3-ligases (CRL4) and functions as an adaptor protein to link Cullin 4A (Cul4A) and protein receptors marked for ubiquitination. The compounds selected in this study would play the role of ligands for recruiting a target protein for an E3 ubiquitin ligases, leading to proteolysis of the target by ubiquitin-proteasome system (UPS).

Ligand structures were selected from the ZINC database on the basis of docking analysis to DDB1 protein by AutoDockVina software. As a part of blind docking, ligand docking was performed to the entire DDB1 molecule excluding binding sites for other protein complexes. The compounds with the lowest binding free energy of DDB1-ligand interactions were selected for the study.

The new type of DDB1 ligands will be use for the synthesis of the new PROTACs (proteolysis target chimeras) class that recognizes a selected pathological (*e.g.* cancerous) protein and directs this protein to ubiquitin-dependent degradation by the ubiquitin-proteasome (UPS) system. The designed PROTAC molecule will be composed of the DDB1 ligand selected in this study, a linker and a ligand that binds to protein targeted for degradation.

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Keywords: DDB1, small molecule ligands, blind docking, PROTAC

P-49: Activity of inhibitors of the two *Helicobacter pylori* purine salvage pathway enzymes (purine nucleoside phosphorylase – PNP and adenylosuccinate synthetase – AdSS) *in vitro* and *in vivo*

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The gram-negative bacterium *H. pylori* is a wide-spread human pathogen responsible for the development of many diseases such as peptic ulcer or gastric cancer. It has been estimated that nearly 50% of the world's population is infected by this pathogen. The rapid increase of *H. pylori* resistance to many antibiotics in recent years has become a major public health concern. Thus, it is important to search for novel targets for antimicrobial drugs. *H. pylori* is one of the microorganisms lacking the *de novo* purine nucleoside synthesis pathway and it can obtain purine nucleosides for the synthesis of DNA and RNA only *via* the purine salvage route. Purine nucleoside phosphorylase (PNP) and adenylosuccinate synthetase (AdSS) are crucial enzymes of this pathway. The purpose of our research was to evaluate the usefulness of both enzymes as possible new drug targets. Hadacidin and formycins A (FA) and B (FB) are known inhibitors of AdSS and PNP, respectively. We have shown that they inhibit 26695 strain *H. pylori* enzymes *in vitro*, with the following inhibition constants: 0.19±0.02 µM for hadacidin vs. AdSS, and 14.0±1.7 µM and 0.96±0.08 µM for FA and FB *vs.* PNP. However, in the *in vivo* experiments these compounds do not affect significantly the growth of *H. pylori* 26695 strain culture; the slight effect was observed only for FA in a very high concentration (5 mM). Therefore, we decided to check their cellular uptake by *H. pylori*. Extracellular concentration of formycins was monitored by LC-MS and UV absorption, while of hadacidin (too small and volatile to be detected by the LC-MS) by measuring inhibition of AdSS. The obtained results confirm that FB at 350 µM and 35 µM, and hadacidin at 350 µM do not penetrate into *H. pylori* cells, while for FA at 350 µM and 35 µM some modest uptake was observed. These data show that there is an urgent need to search for other PNP and AdSS inhibitors to estimate the usefulness of both enzymes as targets for new drug against *H. pylori*.

Keywords: Helicobacter pylori, purine nucleoside phosphorylase, adenylosuccinate synthetase, cellular uptake

P-50: Differentiated murine Neuro-2a as a model to study toxicity of metal nanoparticles

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Introduction: Neuro-2a (ATCC® CCL131™) is a neuroblast cell culture from Mus Musculus. Neuro-2a is neural crest-derived cell line that has been extensively used to study neuronal differentiation, axonal growth, signaling pathways and viral infection. Undifferentiated Neuro-2a cells have neuronal or ameboid morphology. In this study we used treatment with 20mM retinoic acid or forskolin to develop an *in vitro* model to study toxicity of noble metal nanoparticles.

Material and methods: Neuro-2a cell were initially cultures according to the producer's protocol (ATCC). For differentiation, cells were centrifuged for 300 g for 10 minutes and suspended in Neurobasal with 1% B27 supplement. 24 hours before exposition to nanoparticles, forskolin (10mM) or retinoic acid (20mM) were added. Toxicity of 10 and 30 nm AgNPs; 10 and 30nm AuNPs modified with tannic acid suspended in culture media was assessed using MTT and Neutral Red tests in the range of concentrations: 1 – 10 µg/ml with 24h exposure.

Results: Both retinoic acid and forskolin sufficiently differentiated Neuro-2a cells into neurons, although forskolin was more efficient as determined by morphology assessment. When comparing toxicity of undifferentiated and differentiated Neuro-2a culture, we found that toxicity of tannic acid modified nanoparticles was directly related with a degree of neuronal differentiation. While for undifferentiated Neuro-2a $TCID_{50}$ dose was 10 µg/ml, for forskolin-differentiated cells, the $TCID_{50}$ dose was 2.5 µg/ml. In summary, our results prove usefulness of forskolin differentiated Neuro-2a cells in the study of nanoparticle toxicity.

The study was supported by National Science Centre grant no. 2018/31/B/NZ6/02606.

Keywords: neuron, metal nanoparticles, toxicity

P-51: LSD1 activity promotes LPS-induced inflammation of microvascular endothelial cells

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Inflammation is the body's natural response to stress in the broadest sense. The regulatory mechanisms that control this process, some of which are still unclear, are needed to balance the immune response, but also when insufficient, can cause immunodeficiency resulting in infection, cancer, neurodegeneration or other serious disorders. In this study we focused on defining the role of lysine-specific demethylase 1 (LSD1), an enzyme involved in modulating the methylation state of lysine, including histone and non-histone proteins, in shaping the inflammatory profile of endothelial cells (EC). To determine the role of LSD1 in the inflammatory response we observed EC reaction on proinflamatory stimuli (lipopolisaccharide; 100 ng/ml) on two research models. The first was pharmacological model where the activity of LSD1 was inhibited using 2-PCPA (tranylcypromine), the second was the transcription model of LSD1 deficient cells (HMEC-1 LSD1 KD) obtained by lentiviral shRNA infection. Analysis of both experimental models revealed an altered inflammatory response following both LSD1 inhibition and LSD1 silencing. We observed decreased U937 lymphocyte recruitment to activated endothelial cells and decreased extracellular secretion of many NF-κB dependent proinflammatory cytokines, also confirmed at the transcript level by RT-qPCR. Monitoring of the LPS-induced p65 translocation revealed inhibition of the NF-κB subunit in LSD-1 KD *vs* nonTarget (control cells) as well as due to pretreatment of 2-PCPA cells. Gene profiling performed with RNA microarrays confirmed the obtained biochemical data at the transcript level. Overall, the studies carried out show that LSD1 has a proinflammatory effect on endothelial cells.

The research was financed by the National Science Center, project no. 2016/23/N/NZ3/02435.

Keywords: lysine-specific demethylase 1, histone demethylase, inflammatory response, endothelial cells

P-52: Effect of cysteine mutations on the function of the mitochondrial form of the peroxide sensor Gpx3

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Gpx3 is one of the most important antioxidants in yeast and has a homolog, Gpx4, in mammals. It has two isoforms: Gpx3 localised both in the cytosol and mitochondria and N18Gpx3 with an 18-amino acid N-terminal sequence targeting it specifically to mitochondria. Importance and functions of particular residues in N18Gpx3 have not yet been revealed. Here we show, that mutations of cysteine 36 significantly improved H_2O_2 resistance of strain expressing it, whereas the impact of Cys64 mutation was not that clear and changing presequence from N18 to cytochrome b2 mitochondria-targeting presequence combined with mutation of Cys36 increases viability of cells. The N18 presequence turns out to have more functions than targeting to mitochondria as a positive impact of Cys36 mutation was much more explicit in N18Gpx3 than in Gpx3. Both Cys36 and Cys64 mutations decreased H_2O_2 sensitivity in respiratory and fermentative conditions excluding Cys36 in Gpx3 in fermentative conditions. Impact of the latter mutation is the most significant in the H_2O_2 sensitivity. These results show that mutations of important residues in that protein can be beneficial for cells and that N18 presequence has an important effect on the enzymatic activity of glutathione peroxidase. We anticipate that it will be the turning point not only for research on presequence impact on enzyme behaviour but also in research on various isoforms of Gpx3 homologues.

Keywords: mitochondria, biogenesis, Gpx3, Glutathione peroxidase 3, mitochondrial import

P-53: YEAST, WE CAN! The Factory of Extracellular Cellulases for Biotech Applications

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Due to the population growth, greenhouse effect as well as the expansion of industrialization, the renewable energy sector is being widely explored for last few decades. Biotechnologists around the world pay a great attention to the gaseous (biogas), solid (biomass) and liquid (bioethanol, biodiesel) biofuels. Although it is possible to efficiently produce bioethanol using wheat, maize or sugar cane, non-negligible ethical conflict of using edible corps for industrial purposes exists. Promising alternative is hydrolyzed lignocellulosic biomass as fermentation substrate. Nevertheless, chemical hydrolysis unveils a number of new technological problems, making it difficult to apply. Here the enzymatic processes succor. But where do these enzymes come from?

Environmental yeast strains are *the gold mine* according to their unexplored biochemical properties. In this study 23 yeast strains from *WUT Yeast Collection*[1] were tested for cellulases production and other 14 strains for efficient ethanol production.

Cellulolytic properties of yeasts were tested on Petri dishes with medium containing 2% carboxymethylcellulose (CMC). After screening, few strains were selected for further cultivation in a liquid medium, among them *Cystofilobasidium macerans* WUT145 gave astonishing results ($EA = 66.23 \pm 0.15$ µmol×mg^{-1}×min^{-1}) [2].

The investigation revealed strain *Kluyveromyces marxianus* WUT216 as a highly efficient thermotolerant bioethanol producer. Properly preserved fermentation and growth abilities were observed from 15 to 42°C. What is really interesting WUT216 strain was capable to ferment xylose, sugar present in lignocellulose, directly to ethyl alcohol.

Obtained results are very helpful in further investigation towards combined yeasts cultures for simultaneous lignocellulose hydrolysis and bioethanol production.

[1] wutyeastcollection.pw.edu.pl
[2] Chreptowicz *et al. Microorganisms* 2019, 7, 653

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Keywords: cellulases, bioethanol, yeast, WUT Yeast Collection, *K. marxianus*, *C. macerans*

P-54: Identification of a novel Ag-binding peptide and its presentation on the surface of T7 phage capsid

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In 1985, the 2018 Nobel laureate George P. Smith described a M13 *Phage Display* technique. Based on fusion of short nucleotide sequences with the sequence of *pIII* gene of M13, the technique enables the display of various peptides on pIII protein of M13 and construction of a peptide library. With such a peptide library, it is theoretically possible to identify any peptide with a desired feature, e.g. binding to metal or metal oxide nanoparticles.

The aim of this study was to identify a new peptide sequence with high binding efficiency to silver nanoparticles (AgNPs) and to present it on a T7 capsid.

At the beginning, with the use of a M13 phage library and biopanning procedure, we identified a new AgNP-binding peptide (RFEHPAVPRTEM). Next, phage-exposed peptide binding efficiency to AgNPs was explored. The binding efficiency to AgNPs was three orders of magnitude higher in comparison to M13KE (maternal M13 phage). We then constructed T7 phages presenting the identified peptide on their virion surfaces on gp10A protein. The identified peptide (RFEHPAVPRTEM) displayed on T7 did not affect phage virion morphology and such recombinant T7 phages (T7-AgNPs) were effectively propagated and purified.

In conclusion, we constructed a new molecular tool - a recombinant T7-AgNPs phage - that could be used in various experimental efforts including silver nanoparticle synthesis or in biomedical applications.

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Keywords: bacteriophages, phage display, silver, nanoparticles, biopanning

P-55: Impact of copper ions addition on *Miscanthus* × *giganteus* plant regeneration via somatic embryogenesis in callus obtained from immature inflorescences

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Miscanthus × *giganteus* is a perennial C4 grass that is more adapted to temperate climate in comparison to other phylogenetically-related C4 crops such as maize, sorghum or sugarcane. Hence this plant is used for the production of a large amount of biomass, being feedstock for highly calorific pellets and other biofuels. Miscanthus straw is used for production of building materials, in papermaking and many other applications. However, since *M.* × *giganteus* is a hybrid of diploid *M. sinensis* and tetraploid *M. sacchariflorus*, the result triploid genome causes sterility of the species and the possibilities of its reproduction are limited. Therefore, it is advisable to develop an optimal regeneration protocol, both to produce plant seedlings, and as a tool enabling molecular and biological research aimed to increase the potential of *M.* × *giganteus* as a bioenergy crop.

In the study, callus was induced in inflorescences at early stage of development of 4 genotypes of *M.*× *giganteus*: Illinois, D-116, MG3, MG4. We used 8 variants of media, 4 based on MS and 4 based on C17 supplemented with maltose, both with different concentrations of 2,4-D (2.5-5 mg/L), and BAP (0.01-0.5 mg/L) but with a constant concentration of CuSO4 (5µM) Due to the histological images, morphogenic structures formed by round-shaped cells with large nuclei and small vacuoles were observed, from which somatic embryos developed. Non-embryogenic callus was also observed, consisted of elongated and highly vacuolated cells with small nuclei. Plantlets were regenerated after transfer of embryogenic callus on MS-based medium with added NAA (0.5 mg/L) and KIN (0.5 mg/L). There were determined differences in effectiveness of regeneration, including callus formation and somatic embryogenesis, which depended on miscanthus genotype, concentration of 2,4-D and BAP in media and duration of *in vitro* culture.

Keywords: *Miscanthus* x *giganteus*, *in vitro* cultures, somatic embryogenesis, immature inflorescence

P-56: The potato juice as cost-effective medium for biosynthesis of bacterial cellulose

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Bacterial cellulose (BC) is a versatile biopolymer, most effectively synthesized by non-pathogenic *Komagataeibacter xylinus*. BC does not contain lignins, pectins or hemicelluloses. Importantly, biocompatibility, non-toxicity and biodegradability of BC makes this material safe-to-use not only with regard for industrial applications, but also for environment. Thanks to the above-mentioned properties, BC finds versatile applications, e.g. in food, cosmetics, pharmaceutical/biomedical, paper and textile industries. Nevertheless, the high cost of dedicated, microbiological medium used for BC production significantly hinders possibility of its broad application. Searching for alternative, we turned our attention to potato juice (PJ), being substantial leftover of starch industry, and which massive volumes, used as fertilizer, causes water contamination and eutrophication. Therefore, the aim of present research was to verify the possibility of using a cost-effective, ecological-friendly medium made of PJ, in order to obtain BC of properties at least as good as of polymer obtained from commercial medium. According to our method, no PJ's pre-treatment was required prior introduction of BC-forming bacteria. The results of our study indicated that BC yield gained from PJ was comparable to the yield gained from commercial, optimal medium. It was also shown that the cellulose yield depended on the variety and type of potato from which the cell juice was obtained. The performed analyzes of microstructure, physicochemical parameters, chemical composition and cytotoxicity showed no significant differences between investigated vs. control BC. Moreover, BC obtained from PJ did not contain any, hard-to-remove impurities, what is important in process of further polymer's preparation to use. Therefore, the two additional values of present study are possible re-purposing of starch industry left-over and significant drop of costs related with BC production.

Keywords: bacterial cellulose, synthesis, potato juice

P-57: Evaluation of the most efficient isolation method of mesenchymal stem cells (MSCs) from different regions of human umbilical cord for medical treatments

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Umbilical cord (UC) is a good source of mesenchymal stem cells (MSCs) that can potentially be used in clinical treatment. MSCs could be isolated, stored for a prolonged time and used in cell therapy. How successful the use of MSCs is going to be, largely depends on how efficiently they are derived, how well their preparation is optimized and how well they are cryopreserved. To address this issue, we performed a study to find out which area of UC is the best reservoir of MSCs. Accordingly, UCs were cut into 1–2 mm slices. Explants from the selected areas of the UC were obtained using a biopsy punch and transferred to 6-well plates. The selected regions included: the centrally located Wharton's Jelly in between the blood vessels and the border area of the cord. MSCs were also derived from the homogenates of the entire slice. When cultivated in low oxygen conditions (5%), mimicking the physiological normoxia, MSCs migrated from either different explants or homogenates. After being cultured for 5-7 days, the first cells to migrate out into the growing medium were those from the slice homogenates. Subsequently, the Wharton's jelly-derived MSCs revealed their presence. The isolated cells were then passaged and cultured for additional 3 more days. After being trypsinized, the cells were counted, centrifuged, resuspended in a cryopreserving medium and stored in –80°C. The results of the cell count showed that the slice homogenates were the best source of MSCs. These cells, being most numerous, appeared the fastest in the culture in comparison to the cells derived from other areas and they were characterized by the highest proliferation rate. In conclusion, the performed study allowed us to evaluate the most efficient MSCs isolation method, which requires homogenizing the entire slices of a UC. The MSCs isolated from selected regions will be further examined, including testing their ability to form colonies, secrete immunosuppressive factors and protect the nervous system.

Keywords: mesenchymal stem cells, umbilical cord, cell therapy

P-58: New bacteriophages infecting *Bacillus* spp.

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Genus *Bacillus* groups aerobic or facultatively anaerobic gram-positive bacteria. Its members occur in wide range of environments including soil, air and surface of the organisms. Moreover, many of these bacteria produce resilient endospores that facilitate their spread and colonization of new niches. Some of these microorganisms are pathogens of plants or animals, including humans or are involved in the process of food spoilage. For instance, *Bacillus cereus*, cause food poisoning, closely related to *Bacillus anthracis* life – threatening anthrax and *Bacillus thuringiensis* is a pathogen of insects. One can argue that a in order to understand the biology of bacteria, it is important to discover bacteriophages that attack them. During 2 years of the "Viruses in the environment" course above 30 new phages infecting *Bacillus* were isolated. Unfortunately, these viruses have not been characterized yet. The aim of our study was genomic analysis of four selected bacteriophages from the collection (three *B. pumilus* phages and one infecting *B. subtilis*). These viruses were cultured, their DNA was purified, sequenced and assembled. Comparative genomics analysis revealed that three of phages are probably new isolates from of genus *Agatevirus* and the remaining one is likely a salasivirus. To sum up, this project provides new understanding of the genetic diversity of *Bacillus* phages revealing another portion of a great reservoir of yet unexplored genes.

Keywords: bacteriophage, genomic, environmental microbiology

P-59: Fungal battle – pathogenic versus endophytic fungi of ash (*Fraxinus excelsior* L.) in a study on active substances – the weapon production by the endophytes

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The battleground is ash (*Fraxinus excelsior* L.) and the enemy is pathogenic fungi (*Hymenoscyphus fraxineus*), which by infecting ash, causes its dieback. The purpose of the study was to check whether ash endophytes play a role in defeating the pathogen. Current trends in phytopathology focus on using biological agents. One way to fight with *H. fraxineus* may be the use of other organisms present in the ash environment, for example their natural fungal endophytes. In this study, some ash-friendly fungi were isolated from ash branches and leaves and were then tested in dual-cultures with *H. fraxineus*. Six endophytes won first game, indicating the ability to suppress the growth of the pathogen. Thus, some secreted active substances – their secret weapon – may be the reason for this inhibition. It was decided to find them and to verify whether secretion of these metabolites is affected by the presence of the pathogen. This problem was approached by using liquid-culture medium in two experimental setups. Firstly, pathogen was grown with endophytes. Secondly, each endophyte was grown in a supernatant of pathogen culture. In both cases, the contents of media after culturing were analyzed in regards to the their composition after growing each of the fungi separately. The studies were conducted after three weeks of growing using high pressure liquid chromatography (HPLC). A comparative analysis of all supernatants showed that some of tested endophytes secreted different substances when grown alone and when grown in the pathogen supernatant.

Obtained result can help to understand the interactions between studied fungi on their battlefield. Detected metabolites may be used, in the future, to inhibit pathogen growth to stop ash dieback.

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Keywords: fungi, ash dieback, HPLC studies, fungal metabolites

P-60: Optimization of process of cloning and expression of the ice-binding protein isolated from psychrophilic yeast *Glaciozyma*

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Over 80% of Earth's surface is periodically exposed to temperatures below 5°C, including mostly deep sea, permafrost of the Arctic and Antarctica. Despite the conditions, these areas are rich in organisms adapted to cold – psychrophiles. One of its ways of adaptation to low environmental temperatures is production of ice binding proteins (IBPs), including antifreeze proteins (AFPs). AFPs bind to the ice surface and decrease the freezing point of body fluids in organisms to avoid freezing. They can be used in the industry in which the protection from forming bigger ice crystals is essential – including food industry, agriculture and medicine.

The goal of the research was to optimize cloning conditions of *afp* gene to plasmid pGEX-6P-1 and to obtain the expression of the recombinant AFP protein derived from psychrophilic yeast *Glaciozyma antarctica*. Vector pGEX-6P-1 was successfully ligated with *afp* gene, what was later confirmed by DNA sequencing. Transformation of competent cells *E. coli* TOP10F' and BL21 GOLD was done to carry the plasmid with the inserted gene. The *afp* gene was expressed in the expression strain *E. coli* BL21 GOLD and the protein was present in the inclusion bodies, which can lead to further renaturation and purification of the recombinant protein.

To sum up, the activity of AFPs causes formation of smaller ice crystals non-lethal to cells, what makes them have a great potential in industry, for example as cryoprotectants. They are not used on the bigger scale due to its high cost of production, so we should concentrate on increasing their production efficiency. For this reason, it is so important to understand mechanisms of its unique activity and to optimize their expression.

Keywords: psychrophilic yeast, cloning, protein expression, ice-binding proteins

P-61: Platinum nanoparticles and cisplatin: interactions and biological activity of the analyzed substances

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These days, platinum nanoparticles (PtNPs) play critical role not only in different areas of technology but also in medicine [1]. PtNPs are distinguished by unique characteristics like large surface to mass ratio and high reactivity. The size of PtNPs affects its solubility and, as a consequence, bioavability [2]. Platinum nanoparticles can have a crucial role in drug delivery as well as may improve anticancer efficiency or reduce drug resistance [3].

Platinum based anticancer drugs, like cisplatin, are widely used in the treatment of various types of malignances, among others, ovarian, testicular or lung cancers [4,5]. The difficulty is that, despite efficiency, cisplatin may cause various side effects like nephrotoxicity, cardiotoxicity, hepatotoxicity etc. and also leads to drug resistance [3,4].

The main purpose of this study is to investigate the possible integrations between the PtNPs and cisplatin as well as to find out if the interactions could affect the biological activity of the agents.

In this research, there are used two biophysical methods: Dynamic Light Scattering and Isothermal Titration Calorimetry and a biological method – Ames mutagenicity assay on *Salmonella typhimurium* TA102 strain. The experiments enable to test the direct interactions and the biological activity of the analyzed compounds.

The preliminary studies confirmed the existence of the potential interactions between the PtNPs and cisplatin. The biological Ames mutagenicity assay also verify the possible influence of the PtNPs on the mutagenicity of the tested drug.

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Keywords: nanoparticles, platinum nanoparticles, cisplatin, non-covalent interactions, mutagenicity

P-62: Visualization of actin cytoskeleton dynamics, analysis of cell cycle progression and proliferation in dermal fibroblasts derived from wild cats – preliminary results

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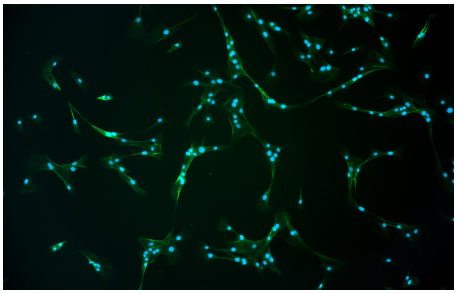
Gene resources are invaluable for conservation, assisted reproduction techniques, evolutionary biology, and wildlife medicine. It is particularly important to establish genetic banks involving somatic cell lines (preferably fibroblasts) to enable use of them to re-establish or expand threatened populations by somatic cloning. Undertaking research focused on the thorough explanation of biological features of dermal fibroblasts is crucial in view of using these cells in somatic cloning.

The study aimed at analysis of cell cycle progression, cell proliferation, and visualization of actin cytoskeleton in dermal fibroblasts derived from 4 wild felids: jaguarundi, pallas cat, jungle cat, and lion. Cell cycle was examined by flow cytometry. Cell proliferation was measured by proliferation index [PI = (S + G2M)/(G0/G1 + S + G2M) x 100%]. For visualization of F-actin filaments and nuclei, cells were stained with phalloidin and DAPI.

Changes in F-actin filaments polymerization, cell shape in coordination with cell cycle progression were noted by imaging. Cells during prophase, interphase, cytokinesis were captured. In jaguarundi, and jungle cat fibroblasts micronuclei-resembling structures were observed. Wild cats' fibroblasts were characterized by a relatively long G0/G1 phase, with a similar proportion of cells at this stage (>80%), and proportion of cells (>10%) at G2/M phase, with exception of lion fibroblasts (6,1%). G2/M checkpoint is key in preventing cells with damaged DNA from undergoing mitosis. The proportion of cells in distinct cell cycle phases (G0/G1, S, G2/M) in jaguarundi was 85.4, 2.6, 12.0; pallas cat 83.6, 4.5, 11.9; jungle cat 81.4, 2.6, 16.0; lion 87.2, 6.7, 6.1, respectively. The highest PI (%), which corresponds to a rapid cell proliferation was observed in jungle cat fibroblasts (18.6), the lowest in lion (12.8). In jaguarundi and pallas cat PI was 14.6, 16.4, respectively. This research provides preliminary insight into wild cats' fibroblasts biology.

Keywords: dermal fibroblasts, cytoskeleton, cell cycle, cell proliferation, wild cats



P-63: Targeting proteins for degradation in bacteria: a new approach for antibiotic discovery and functional study of protein functions

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Targeted degradation of proteins is gaining popularity in drug development research, especially in such fields as oncology. Proteolysis-targeting chimeras (PROTACs) are eukaryotic heterobifunctional molecules comprised of two ligands joined with a flexible linker, that target proteins for degradation by recruiting E3 ubiquitin ligase, which results in ubiquitination of the target protein and its degradation in 26S proteasome. In bacteria such approach cannot be implemented, since they lack ubiquitin-proteasome system, however they do contain numerous other degradation machineries that could be taken into consideration. The one of our particular interest is the bacterial AAA+ family protease complex ClpXP, the main protease responsible for ssrA-tagged protein degradation. Our idea is to develop molecules (degraders) consisting of peptides or/and small molecules linking the part capable of recruiting and activating the protease (e.g. degrons based on ssrA tag, ADEPs) with ligands that specifically bind target proteins. We created solely peptidic degraders, encoded on the expression plasmids with the inducible promoter and transformed them into *Escherichia coli*, the model organism in which we will test their effectiveness by studying the phenotype or immunoblotting. As a further validation, we will test the affinity of degraders to their purified targets as well as their effect on protease activity *in vitro*. We assess the effectiveness of degrons by studying tagged eGFP stability in *E. coli* using fluorescence measurements. Development of effective tools for targeted proteolysis in bacteria could provide a novel method for studying protein functions *in vivo*, which lacks the limitations of recent reverse genetics approaches. Additionally, degraders could also be used as a new class of antibacterial agents in the post-antibiotic era.

Keywords: proteolysis, TPD, antibacterials, ClpXP, ssrA

P-64: Colocalization of New Coumarin-based Fluorescent Probes Using LysoTracker Red and MitoTracker Red probes

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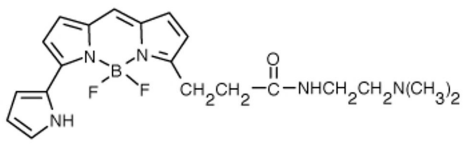
Fluorescence bioimaging is an excellent tool for analyzing internal cellular structures and observing dynamic processes occurring in eukaryotic cells using fluorescent dyes. Desirable characteristics of fluorescent dyes include the ability to penetrate into cells, high fluorescence efficiency and selectivity in the staining process. This enables clear, unambiguous visualization and location of structures inside the cells. In addition, these compounds should not be cytotoxic in order to be used safely on cells. Coumarin derivatives were examined spectroscopically for use in imaging of eukaryotic cells in fluorescence microscopy. A non-small cell lung cancer line (A549) was used during the study. Analysis performed with the Leica DMiL LED Fluo inverted microscope. These compounds have been shown to be effective as fluorescent dyes. LysoTracker and MitoTracker Red are fluorescent probes widely used for viable cell staining of lysosomes and mitochondria, respectively. The molecular structures of LysoTracker Red (Figure A) and MitoTracker Red (Figure B). These commercial probes were used in colocalization studies of novel coumarin derivatives. The partial localization of new coumarin derivatives in lysosomes was confirmed. In addition, the effect of coumarin derivative compounds on the cytotoxicity of cells was checked. For this purpose, a colorimetric MTT test was used. Cytotoxicity of the tested compounds was compared with the doxorubicin compound, which has proven toxicity to cancer cells. After the MTT test, it was shown that the coumarin derivatives tested are safe for cells and do not affect their physiological properties after both 3 and 24 hours of incubation.

Acknowledgements: The authors are grateful to the Foundation for Polish Science (Warsaw, Poland) – Project POWROTY (Contract No. POIR.04.04.00-00-1E42/16-00-POWROTY/2016-1/4 – "Synthesis and photochemistry/photophysics studies of the intelligent luminescent molecular sensors for selective detection in biochemistry and chemistry").

Keywords: coumarin, fluorescent probes, colocalization, LysoTracker Red, MitoTracker Red

A



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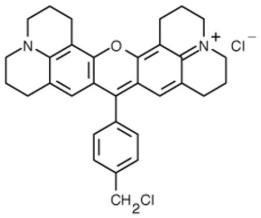

P-65: Inexpensive and simple ways to miniaturize electrified liquid/liquid interface

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The interface between two immiscible electrolyte solutions (ITIES) holds a special place in electrochemistry. Sensors based on ITIES are not restricted to oxidation or reduction reactions of the analyte, which allows the analysis of chemical species that are undetectable with traditional electrochemical methods [1-3]. In other words, the detection mechanism can originate from the ion transfer across the liquid - liquid interface.

ITIES systems can be easily miniaturized, so that additional analytical benefits can be achieved. The main points to be considered are higher sensitivity and lower detection limits. Consequently, the development of nano- or micro-ITIES is attracting a lot of attention.

In this work, we describe protocols allowing for simple miniaturization of polarized liquid/liquid interface. First approach is based on the fiberglass membranes decorated with a polyelectrolyte multilayer using the Layer-by-Layer (LbL) assembly. Second protocol is based on a microholes punched in a self-adhesive polyamide film. For the third miniaturization protocol very thin aluminum foil was used. All platforms were used as a support for the LLI and were characterized using ion transfer voltammetry in the presence of quaternary ammonium cations.

Acknowledgments: The presented research was financed by the National Science Center Poland as part of the SONATA 15 project (UMO-2018/31/D/ST4/03259).

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Keywords: ITIES, electrochemistry, miniaturization, voltammetry

P-66: Optimizing the multi-cell isolation process to approach the real-life interactions of skin cells *in vitro*

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Each body tissue is composed of various cell types. A multi-cell isolation process is particularly important in many experimental studies *in vitro* targeted at the understanding of tissue homeostasis and interactions occurring between different human cells present in each tissue. The use of numerous cell types isolated from the same tissue allows for a better presentation of the real interactions occurring between human cells than with the use of only commercially available cells obtained from different donors and then frequently immortalized. An effective isolation of several cell types from the same tissue would facilitate experimental research on the real-cell interactions *in vitro*.

The research object of this investigation is human skin and the study aim was to develop the best protocols for the simultaneous isolation of primary skin cells from epidermis (keratinocytes and melanocytes) and dermis (fibroblasts and mast cells) from skin obtained from plastic surgery. Understanding problems and limitations of each step of the cell isolation process is important to improve the existing protocols and increase the efficiency of the process. The ones occurring during this research will be presented and discussed in detail. The optimized isolation procedure consists of several steps of mechanical and/or enzymatic character, which vary depending on the cell type. Next, cell culture origin was confirmed by the qPCR technique. Furthermore, each cell type was stained with a fluorescent tracer and various cocultures were carried out for microscopic observations of the real-time interactions *in vitro*.

In conclusion, the presented multi-cell isolation process of different skin cells can be of a valuable help for other researchers, who wish to study real-cell interactions *in vitro* and want to establish their own protocol of cell isolation.

Acknowledgements: This research was financed from the BIOTECHMED-2 start project provided by the Warsaw University of Technology under the program Excellence Initiative: Research University.

Keywords: multi-cell isolation, real-life interaction, skin cells, mast cells, *in vitro*

P-67: The application of ion transfer voltammetry in determination of phenylethylamine in milk samples

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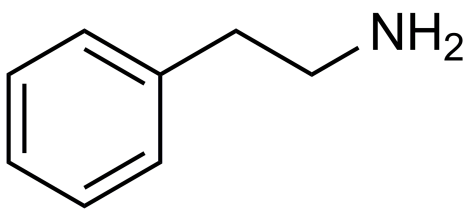
Phenylethylamine (PEA) is an organic compound classified as a biogenic amine. It fulfills its role as a neurotransmitter, a component of many drugs, hormones, and intoxicating substances, it is also found in food. Its highest concentration is noted in stale food, and its excessive intake may cause serious disturbances in the functioning of the body. Therefore, the content of PEA in food products should be strictly controlled.

In the present work, ion transfer voltammetry (ITV) was used to investigate phenylethylamine (PEA) at the polarized liquid – liquid interface in a milk sample. Electrochemistry at soft junctions (interface between two immiscible electrolyte solutions, ITIES) is a branch of electrochemistry that describes phenomena at the polarized boundaries of two immiscible liquids. Reactions occurring at the ITIES can be reversible with respect to a simple interfacial ion transfer. The electrochemical testing of PEA using ITIES has never been described in the literature before. In our research, the concentration dependence of PEA was studied with the focus on spoiled milk samples.

Keywords: ITIES, food, electrochemistry, PEA



P-68: Antimicrobial potential of St. John's wort plant extracts to various test microorganisms

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The discovery of antibiotics and their application in medicine is one of the greatest discoveries in humanity. Thanks to their wide application and use the battle against many diseases and infections caused by various microorganisms has been adopted. Today humans are facing an increasing number microorganisms that are resistant to antibiotic treatment. The discovery of new potential drugs against the growing number of resistant species is one of the greatest problems today. From this global problem arises the goal of this project, finding new, potential drug, with antimicrobial properties, against a wider range of different microorganisms.

In this project, seven transgenic shoots extracts of St. John's wort and their antimicrobial potential against nine different microorganisms were tested and examined. The *Hypericum perforatum* HR shoots extracts were named as: 13 PA, 14 PB, 15 PC, 16 PD, 17 PE, 18 PF and 19 PG. The used test microorganism were: *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* FNS YCC 1, *Rhodotorula* sp., *Aspergillus niger* ATCC 16404, *Penicillium* sp., *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus* sp. and *Staphylococcus aureus* ATCC 6538. The method of microdilution analysis was used, based on the use of microtiter plates and resazurin as an indicator of microbial growth.

The end results showed that *Saccharomyces cerevisiae* FNS YCC 1 (MIC/MMC values 1.56 mg/ml) and *Bacillus* sp. (MIC/MMC values 1.56 mg/ml) were the most sensitive test microorganisms, while *Staphylococcus aureus* ATCC 6538 (MIC/MMC values 12.5 mg/ml) was the most resistant of the treatment with *H.perforatum* extracts. The extracts 13 PA, 15 PC and 17 PE showed the most powerful inhibitory and microbicidal activity.

Final results indicate that these extracts have great antimicrobial action and that St. John's wort as a natural source of these extracts is one of the potential candidates for new, successful drugs in medicine.

Keywords: antibacterial effects, antifungal effects, *Hypericum perforatum*, antibiotic resistance

P-69: We can't use fluorescein diacetate for diatom viability assessment! Or can we?

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Phaeodactylum tricornutum is a marine diatom commonly used as a model organism in molecular biology. This species is strongly predestined for that purpose by low nutritional requirements and the fact that its genome has been sequenced. Though *P. tricornutum* is an object of much research, there are not many suitable viability assays available for that organism. Fluorescein diacetate (FDA) test is frequently used in eukaryotic cells, however the attempts of using it for *P. tricornutum* were not successful as far.

The aim of this work was to overcome the obstacles and create a protocol for viability assay with FDA in *P. tricornutum* using automatic cell counter with fluorescence microscope. Heat was used to induce cell death. For comparison, MTT viability test and maximum quantum yield of photosystem II measurement were used.

Obtained results of FDA test in cultures not treated with heat demonstrated presence of cells showing green fluorescence, what was not observed in heat treated cultures. In cultures where green fluorescence in cells was observed, other methods confirmed the presence of physiological activity. Our study explained possible causes of previous failures in FDA test application and enabled to develop optimal method for FDA viability test in *P. tricornutum*. Presented results prove that FDA test may be useful in *P. tricornutum* under examined conditions.

Keywords: *Phaeodactylum tricornutum*, viability assay, FDA, fluorescence microscopy

P-70: Research on cryptophycin production by cyanobacteria *Nostoc* sp.

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As the number of global cancer cases increases, scientists look for more effective treatments to cure this disease. The new technology of creating antibody-drug conjugates (ADCs) has been studied extensively and proved to be an effective tool to fight against various cancers. The ability of conjugating cytotoxic substances to specific antibodies capable of binding to overexpressed antigens of cancerous cells not only helped to create new drugs but thanks to its working mechanism opened the gate to the usage of stronger, more toxic compounds by reducing side effects of the therapy.

Cryptophycins, cytostatic compounds produced by cyanobacteria of genus *Nostoc* being an example. While displaying great results in killing cancer cells, failed clinical trials due to their high toxicity and many side effects. Cryptophycins are potent tubulin inhibitors that show much higher activity than payloads for ADCs currently used in medicine. Moreover, they show resistance to the most common neutralization mechanism for ADCs which makes them perfect candidates for payload molecules. Though frequently studied, cryptophycin acquisition faces a large impediment in the form of mixed, inconsistent production results.

The presented research focuses on the utilization of *Nostoc* sp. as a reliable source of cryptophycin by investigating the influence of process conditions (light, mixing, additional carbon source) on its stable and repeatable production as well as biomass growth. For example, cryptophycin production seems to be impaired by dynamic conditions of growth (mixing), as well as reacting differently to various lighting conditions and additional carbon source present in the growth medium.

Keywords: cryptophycin, *Nostoc*, cancer

P-71: The role of Ubn2 protein in transcriptional repression of the Hedgehog pathway

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Improper activation and repression of the Hedgehog pathway results in severe birth defects and cancers. Hh signaling regulates the transcription of target genes through three proteins from the glioma-associated oncogene (Gli) family. Gli1 and Gli2 proteins are known as transcriptional activators but the role of Gli3 protein is poorly studied. It is known that it acts mostly as transcriptional repressor in its C-terminally truncated Gli3R form. Whereas the mechanisms of target gene activation by Gli1 and Gli2 are well studied, our understanding of how Gli3R inhibits target gene expression is incomplete. Data from a recent genome-wide loss of function screen based on CRISPR/Cas9 suggests that the Hira protein may negatively regulate Hh-mediated transcription. We identify one of the components of the Hira complex, ubinuclein-2 (Ubn2) as an interactor of Gli3R and investigate the role of Ubn2 in Gli3R-dependent transcriptional repression. This study aims to provide a better understanding of Hh pathway regulation and may help identify new potential therapeutic targets for disorders related to this pathway.

Keywords: hedgehog, cancer, Ubn2, transcriptional repression

P-72: Adalimumab changes expression of TGFβ1-3 in keratinocytes treated with LPS

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Background: One of the drug used in psoriasis therapy is the tumor necrosis factor alpha (TNF-α) inhibitor—adalimumab.

Aim: The goal of this study was to assessment changes in expression of transforming growth factor β 1-3 (TGFβ1-3) in human keratinocyte (HaCaT) culture exposed to lipopolysaccharide A (LPS).

Material and methods: HaCaT was exposed to 1 ng/ml LPS for 8 hours and next for 8 μg/ml adalimumab for 2, 8, and 24 hours in comparison with a control culture. Microarray, RTqPCR and ELISA assay were used.

Results: It was observed changes in expression of TGF β 1-3 as follows: (*TGF β* 1: H-2 vs C +4.16; H8 vs C -8.36; H-24 vs C -11.07; *TGF β 2*: H-2 vs C -3.28; H-8 vs C -4.45; H-24 vs C -3.02;*TGF β 3*: H2 vs C -1.22; H-8 vs C +3.16; H-24 vs C +3.22).

Conclusion: TGFβ1 might be a consider as a supplementary molecular marker useful in monitoring of efficacy adalimumab therapy.

Keywords: adalimumab, keratinocyte, TNF-α, TGFβ, molecular marker

P-73: Boosting with yeast, or how to get diatoms to make more goodies

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Fucoxanthin is a well-known beneficial pigment produced by *Phaeodactylum tricornutum* diatoms. It was shown to function as an anti-cancer and anti-inflammatory pigment. It also possesses additional pro-health properties. Apart from that, *P. tricornutum* cells contain other pigments, such as diadinoxanthin and diatoxanthin, which can also exhibit advantageous functions. Thus, finding more efficient and cost-effective ways to grow these diatoms is an important task of applicative science.

In this study, we checked how the addition of spent yeast sludge from the brewing process can influence the growth of *P. tricornutum* cells and their production of beneficial pigments. To achieve this, we supplemented the standard f/2 medium with the freeze-dried sludge recovered after the final fermentation of beer with US-05 yeast. This dried sludge, containing mostly yeasts and traces of other additions, such as hops, was added to the medium in the amount of either 1 or 2 g/L. Control diatoms were grown without such supplementation.

The addition of spent yeast sludge significantly increased the number of diatom cells already after one week. After 3 weeks, the diatoms cultured with yeast supplementation grew the better the bigger the addition of yeasts was. In the case of 2 g/L addition, they reached two-fold increase in cell count in comparison with the control. Moreover, the size of diatom cells grown with supplementation was significantly larger than in control. In addition, production yields of fucoxanthin and diadinoxanthin were significantly greater when yeast sludge was added to the culture. The diatoxanthin yield depended on the illumination state of diatom culture prior to harvesting.

The obtained result show that post-brewing sludge can be an efficient and low-cost supplement for *P. tricornutum* cultures since it can greatly increase the production of beneficial compounds.

Keywords: diatoms, pigment production, yeast

P-74: Alone we can do so little, together we can do so much – breast cancer cells and fibroblasts clusters in metastasis process

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Breast cancer commonly occurs among women around the world. Its late diagnosis can decrease chances of survival and increase the risk of metastases. Circulating tumour cells (CTCs) mediate metastases formation by detaching from tumour mass and entering blood. In the circulation CTCs are exposed to many unfavourable external factors such as shear stress or immune surveillance, what can reduce their viability. CTCs in order to overcome this problem might form cellular clusters. They can be homotypic (only cancer cells present) or heterotypic (cancer cells plus other type of cells, but mostly cancer associated fibroblasts, CAFs). Main benefits of CAFs in clusters are promotion of cancer invasion, dissemination and increase of CTCs viability both in blood vessels and in metastatic sites, what is crucial for metastases.

The aim of this study was to test if an optimised method for CTCs isolation from breast cancer patients is suitable for obtaining circulating CAFs/CTCs clusters and their further transcriptomic profiling.

In order to do that we developed a method of CAFs/cancer cells cluster formation and in spike-in experiments evaluated stability of the formed clusters at different stages of the procedure. Furthermore, we compared if there were any changes in expression of CAFs marker genes (α-SMA, FAP, CXCL12) before and after isolation procedure as well as after storage of cells in –80°C. Briefly, single cells isolated via micromanipulation were subjected to reverse transcription, preamplification and gene expression analysis using qPCR. Results showed, that density gradient centrifugation does not result in disaggregation of the clusters, but downstream processing significantly decreased the proportion of cells in clusters. Nevertheless, single CAFs could be isolated and profiled for expression of CAFs markers. Further transcriptomic profiling of picked single cells is undergoing.

Keywords: metastases, circulating tumour cells (CTCs), cancer-associated fibroblasts (CAFs), CTC clusters

P-75: Effect of IGH enhancers inhibition with compound 30666 on B-cell lymphoma survival and oncogene expression

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Characteristic feature of B-cell non-Hodgkin lymphomas (NHL) are recurrent translocations placing oncogenes (e.g. MYC) under the regulation of immunoglobulin heavy chain (IGH) enhancers: Eμ, 3'RR1 and 3'RR2. Survival and proliferation of many lymphoma cells depends on the expression of translocated oncogenes. Targeting IGH enhancers could offer a therapeutic strategy for IGH translocation-positive malignancies. Recently, a small molecule 7-[[(4-methyl-2-pyridinyl)amino](2-pyridinyl)methyl]-8-quinolinol (compound 30666) was shown to inhibit Eμ activity, reducing oncogene expression and survival of multiple myeloma and some NHL cell lines. We aimed to validate the inhibitory effect of 30666 in diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) cells carrying the t(14;18) IGH/BCL2 and t(8;14) MYC/IGH translocations, respectively. We established inhibitory concentration 50 (IC50) of compound 30666 in our cell lines, upon treatment for 48h. Next, we tested the effect on expression of translocated oncogene and eRNA derived from Eμ and 3'RR. Indeed, compound 30666 inhibited survival of DLBCL and BL cells with IC50 values 1-5 μM. We observed decreased expression of MYC and Eμ eRNAs in BL cells, but in DLBCL cells BCL2 expression was not affected. 3'RR enhancers responded differently to 30666; 3'RR1 was upregulated and 3'RR2 downregulated consistently in all cell lines. We also tested survival of several IGH translocation-negative B cells and non B cells, where IGH enhancers are not active. We found that majority of them are susceptible to 30666 at similar doses as lymphoma cells. There were no statistically significant differences between t(+) lymphomas, t(-) B cells and t(-) non-B cell groups. Our findings suggest that 30666 inhibitor might not be as specific for IGH enhancers as previously reported and that further research regarding its mechanism of action is necessary.

Funding: Foundation for Polish Science grant no. POIR.04.04.00-00-5EC2/18-00

Keywords: IGH enhancers, eRNA, B-cell lymphoma, cancer, MYC

P-76: The role of sRNAs molecules in an antirepressor-mediated initiation of the phage lytic cycle

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Recently discovered microRNA-type molecules, named UpRoi1 (29-nt long) and UpRoi2 (39-nt long), come from Shiga toxin-converting bacteriophage $\Phi 24_B$. Bioinformatic analyses indicated many binding sites for these molecules, not only in the genome of bacteriophage but also of the *Escherichia coli* host. They occur within bacterial genes coding for important proteins e.g. inner membrane protein or ABC transporter ATPase. In turn, the predicted binding sites of phage origin are localized mainly in genes encoding antirepressors like D_ant, Roi, Cro and Ant, which are essential during phage switch from lysogenic to lytic development. In addition, the analyses showed that UpRoi1 an UpRoi2 could be both *trans*-acting and *cis*-acting sRNAs molecules. In order to verify the supposed role of these molecules in the regulation of the phage lytic cycle, we decided to experimentally investigate the phage development in bacteria. We observed that overexpression of UpRoi 1 and 2 decreased the growth of *E. coli* culture and also the efficiency of bacteria lysogenization with phage $\Phi 24_B$. Importantly experimental results overlap with bioinformatic analyses, and suggest role of UpRoi molecules in the antirepressor-mediated $\Phi 24_B$ prophage induction and its further lytic development in *E. coli* bacteria. At this stage of research, we cannot exclude that UpRoi1 and UpRoi2 may also regulate many bacterial transcripts, however, this requires further confirmation. To our knowledge, these molecules are one of the first candidates of functional microRNA-type molecules of phage origin.

Keywords: microRNA, gene expression, antirepressors, Shiga toxin, bacteriophages