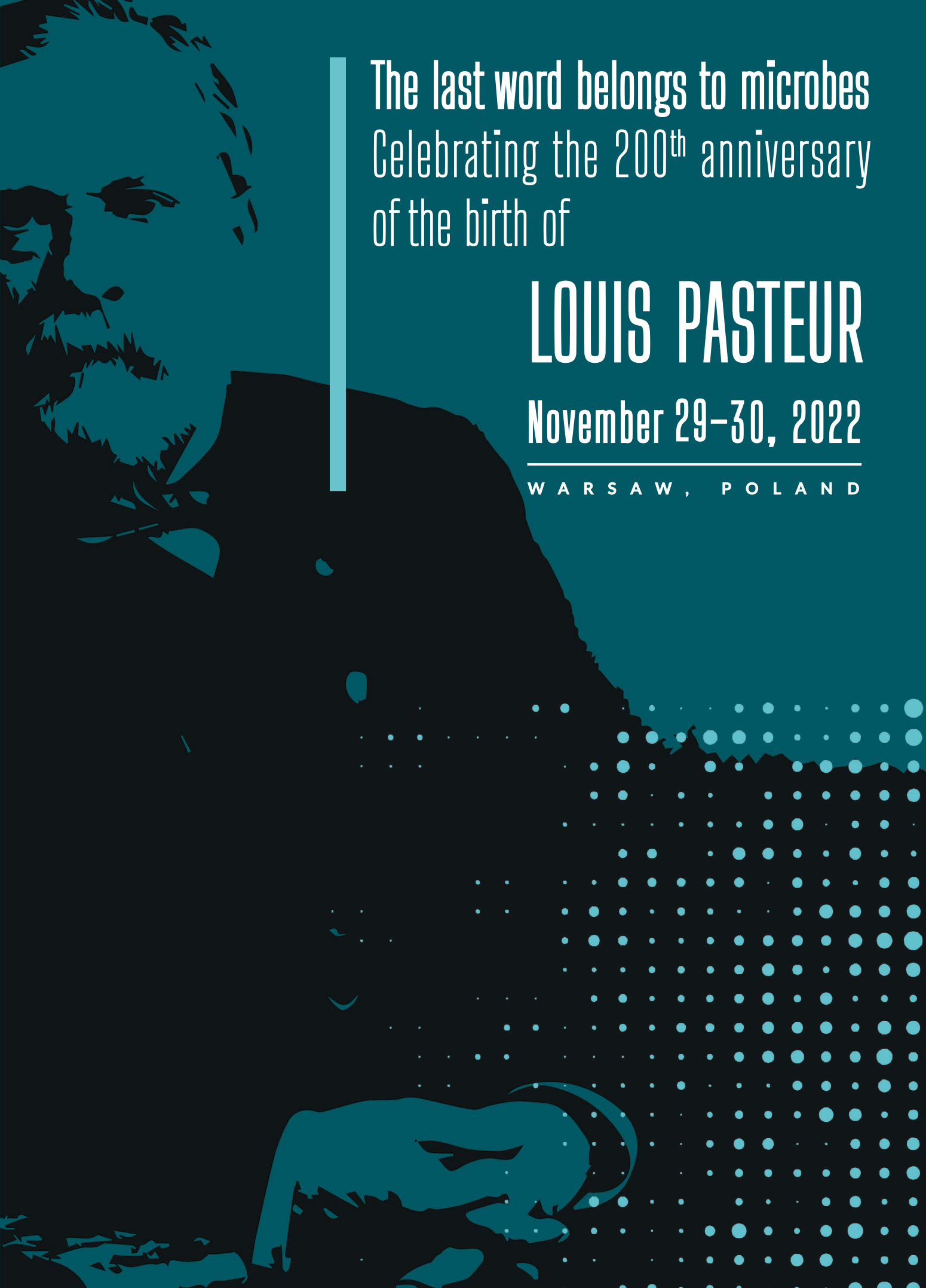

The last word belongs to microbes

Celebrating the 200th anniversary of the birth of

LOUIS PASTEUR

November 29–30, 2022

WARSAW, POLAND

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Celebrating the 200th anniversary
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Contents

Chairman of the Organizing Committee Welcome Letter

Dear Colleagues,

This year we celebrate the 200th anniversary of the birth of Louis Pasteur, the scientist whose name needs no introduction. The life story of Louis Pasteur and his groundbreaking discoveries have earned him a hallowed place in the pantheon of the most illustrious scientific luminaries of the modern era.

Already during his lifetime, Pasteur received worldwide fame and recognition among the scientific community, and more importantly, the heartfelt appreciation and sincere gratitude of millions of ordinary people.

The “father of microbiology”, the “founder of modern vaccinology”, and probably the most meaningful – the “benefactor of humanity” (bienfaiteur de l’humanité) are some of the titles Louis Pasteur is remembered by today. The contributions of Pasteur to the development of natural sciences, and more generally to the development of scientific concepts explaining the essence of life, are outstanding.

Pasteur’s life was a path of epoch-making discoveries in the fields of chemistry, biology, and medicine, and at the same time a paradigmatic example of genuine scientific intuition, research inquisitiveness, and determination.

This year’s bicentennial jubilee of Pasteur’s birth provides a unique opportunity to commemorate the life and achievements of one of the greatest minds of all time.

It is therefore my great pleasure and privilege to invite you, on behalf of the Organizing Committee, to the International Conference “The last word belongs to microbes”, a unique and globally significant event to mark the 200th anniversary of the birth of Louis Pasteur.

The purpose of the Conference is to bring back the memory of Louis Pasteur not only as a true scientist, who bequeathed remarkable legacy of pioneering concepts and fundamental

discoveries, but also as a man whose personal life, character, beliefs, and values epitomise the canonic ethos of science that has continued to inspire and motivate people of all scientific professions and beyond. During the Conference, the life and work of Pasteur will constitute a particularly favorable context for discussing the current state-of-the-art and progress in microbiological sciences towards the end of the first quarter of the 21st century.

With the advent of advanced technologies allowing for the genetic exploration and manipulation of microorganisms, microbiology has entered a new golden age, rendering a plethora of applications for human welfare. Today, this golden age of microbiology faces a broad spectrum of challenges, the most pressing of which are increased antibiotic resistance and (re-)emerging infectious diseases. The COVID-19 pandemic has emphasized more than ever the pivotal role of microbiologists in addressing and curtailing such global health crises.

It is the intention of the organizers to bring together microbiologists from different parts of the world, representing various disciplines, career stages, and institutional backgrounds. The Conference will thus host renowned senior experts, top global figures in their fields, as well as highly talented and productive early- and mid-career scientists who have been involved in wide-ranging and innovative research projects. Many of the invited speakers come from the Pasteur Institute and other research centers affiliated with the global Pasteur Institute network. This preference was given deliberately, since the Institute, founded by Pasteur in the dusk of his life, his last opus magnum, summarizes Pasteur's entire prolific life and stands as a testament of his stellar work for all his successors.

The list of invited speakers reflects the scientific excellence of the conference programme, which covers a breadth of topics including microbial ecology, metabolism, genetics, and pathogenesis, along with antimicrobial drug and vaccine development. Our goal is to make the Conference a scientifically rewarding, professionally and socially invigorating enterprise, with an interdisciplinary and multigenerational environment, which will provide the best forum for stimulating discussions, confronting and exchanging ideas, and forging new collaborations.

The Conference will be held in Warsaw, the capital of Poland, once called "Paris of the North", a city with seven-century tradition and rich and heroic history. I hope you will have a chance to explore the historical and cultural sights offered by the city of Warsaw.

Once again, I cordially invite you to join us and celebrate the 200th birthday of Louis Pasteur!

Tomasz Jagielski
Chairman of the Organizing Committee

President of the Institut Pasteur Welcome Letter

Dear Friends of Louis Pasteur,

We celebrate this year the 200th anniversary of the birth of Louis Pasteur, a global figure in scientific and medical research, a co-founder of the field of microbiology and a pioneer in global health.

His scientific work and thinking about how research should be conducted and its impact on society led to what would become a great scientific movement. The "Pasteur ethos" continues to inspire scientists at the Institut Pasteur, across the Pasteur Network and all over the world.

This jubilee provides a unique opportunity to commemorate the life and legacy of Louis Pasteur who once said: *"Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world"*.

In today's world, let us celebrate Louis Pasteur's scientific and humanitarian values. I wish you all an excellent jubilee conference.

Prof. Sir Stewart Cole
President of the Institut Pasteur

President of FEMS Welcome Letter

Dear Attendees of the Pasteur Jubilee Conference,

I am honored and very pleased to welcome you at the Pasteur Jubilee Conference in Warsaw as we celebrate the 200th anniversary of the birth of Louis Pasteur. Louis Pasteur was one of the greatest microbiologists of all time. His work was extremely important for the development of microbiology and the health and wellbeing of mankind. So, I am very pleased with this initiative in Warsaw. I hope that the work of Pasteur can remind us of the world changing potential of microbiology – subject that all of us share the deepest passion for. I welcome you at this conference in my capacity as the President of the Federation of European Microbiological Societies (FEMS) and the Patron of this conference. The Polish Society of Microbiologists is a valued Member Societies of FEMS, and the Polish Microbiologists are our highly respected colleagues. FEMS is honoured to act as a Patron for this Jubilee Conference and has supported and promoted it via our website and social media activities. Under the grand umbrella of FEMS, we can work to advance and unify the goals of microbiologists and Microbiological Societies across the world. From the very start of my career as an academic, I have been involved in learned societies. I always emphasized the importance of participating in learned societies to my research group. Being involved in senior roles in these societies, including being the President of the Microbiology Society and of ISME, has educated and motivated me greatly. What drives me onwards as President, is working closely with the member societies of the Federation as there is such a variety of sizes and resources available from them.

Many of the microbiologists within the Federation are early career researchers. I have been frequently asked to speak to groups of early career researchers, for example about my own journey and also about what advice I might give to them. I try to encourage those who, like me, are the first in their families to go to university, to study for a PhD and onwards throughout an academic

career. I am passionate about widening participation and raising awareness of the need for diversity in our profession to help solve some of the most pressing problems globally. I am keen to make a plea for further volunteers, either at an individual learned society level or a Federation, such as FEMS. To my mind, volunteering opens up a different world that you may not have known otherwise. You have the opportunity to acquire new skills and I have found that these have enriched my 'day job' greatly. Please, do think about stepping up in a learned society; I have found it to be a very rewarding experience and it has greatly helped me to grow my network too! So, on this momentous anniversary, we gather here to celebrate the birth of a world changing microbiologist, Louis Pasteur. I hope that we can draw inspiration from his example, as we turn our gaze to the future of our discipline and encourage microbiology to grow into all of its potential, diversity, impact, and brilliance.

Hilary Lappin-Scott
President of FEMS

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Conference Programme

Monday, November 28, 2022

12:00–18:00	Arrival and check-in of guests and other participants
18:00	Speakers & Guests Dinner – invitation only

Tuesday, November 29, 2022

8:00	Registration (*Entrance Hall*)
8:30–9:15	Opening ceremony
9:15–9:30	The Institut Pasteur today and the legacy of Louis Pasteur – *Stewart Cole*
9:30–10:00	Louis Pasteur, a child of the Jura, a man for the world – *Daniel Raichvarg*
10:00–12:00	**Plenary Session – PATHOGENS**
10:00–10:20	How the food borne bacterial pathogen *Listeria monocytogenes* promoted the emerging discipline "Cellular microbiology" – *Pascale Cossart*
10:20–10:40	Outsmarting the host: *Listeria* interplay with host cells and tissues – *Marc Lecuit*
10:40–11:00	Control of the saprophyte-to-pathogen transition in *Listeria*: mechanism and clinical implications – *José A. Vázquez-Boland*
11:00–11:20	Interplay between stress and drug resistance in pathogenicity of *Cryptococcus neoformans* – *Łukasz Kozubowski*
11:20–11:40	New developments in rabies post-exposure prohylaxis and therapy – *Hervé Bourhy*
11:40–12:00	Understanding immune evasion by a lethal fungal pathogen – *Robin May*
12:00–12:30	Coffee break

12:30–14:00 **Plenary Session – ANIMALS**

12:30–12:50 The challenge of antimicrobial resistances on the animal-human-environment interface – *Uwe Rösler*

12:50–13:10 Animals as sentinels for environmental fungal pathogens – *Patrizia Danesi*

13:10–13:30 **Gold Sponsor Forum**

- Supporting your microbes – how Th. Geyer offer can make your lab get fit for growth – *Paweł Stępniak*

13:30–14:00 **Silver Sponsor Forum**

- The usefulness of automated chromatography in protein purification – *Stephan Pötsch*
- Looking inside microbiology with Illumina next-generation sequencing – *Piotr Kędziorski*
- Introduction to Corning Life Science – *Marcel Beckert*

14:00–15:00 Lunch break

15:00–16:40 **Plenary Session – SYSTEMS**

15:00–15:20 From pure culture to multi-species communities: contributions of the biofilm concept to bacteriology – *Jean-Marc Ghigo*

15:20–15:40 The potential of long-read sequencing and bioinformatics for fungal and algal genome projects – *Daniel Wibberg*

15:40–16:00 Virological surveillance during sanitary crises: sequencing tools and emergence detection – *Jean-Claude Manuguerra*

16:00–16:20 From smear microscopy to whole genome sequencing: challenges of the laboratory diagnosis of tuberculosis in the 21st century – *Miguel Viveiros*

16:20–16:40 Software tools and databases developed at the Institut Pasteur de la Guadeloupe to map global circulation of *Mycobacterium tuberculosis* complex genotypes – *David Couvin*

16:40–17:00 Coffee break

17:00–18:00 **Plenary Session – THERAPY**

17:00–17:20 Phage therapy: present and future – *Andrzej Górski*

17:20–17:40 Development of novel anti-tuberculosis agents – *Violeta Valcheva*

17:40–18:00 Fungi always have the last laugh: medical advances spoiled by fungal disease – *Ilan Schwartz*

18:00–18:30 **Silver Sponsor Forum**

- New diagnostics trends in molecular microbiology – *Kasper Ciepluch*
- Microbiology in High-Content Screening images – modern assays with microorganisms – *Marek Michałowski*
- Bacteriophage cocktails – a new sustainable solution for infectious agents – *Elżbieta Fornal*

20:00 Gala Dinner – invitation only

Wednesday, November 30, 2022

8:30–11:00 **Plenary Session – ENGINEERING & UBIQUITY**

8:30–8:50 Major evolutionary trends in clinical fungi – *Sybren de Hoog*

8:50–9:10 Evolution of *Mycobacterium tuberculosis* complex – *Roland Brosch*

9:10–9:30 Mechanisms of solvent tolerance in *Pseudomonas* – *Juan-Luis Ramos*

9:30–9:50 Cytosolic bacteria as platforms for innate immune signalling – *Michał Wandel*

9:50–10:10 The predominant clonal evolution (PCE) model of microbial pathogens and its practical relevance – *Michel Tibayrenc*

10:10–10:30 **Gold Sponsor Forum**

- The role of education in building awareness of infectious diseases among doctors and patients – *Paweł Pacholczyk*

10:30–11:30 Best Abstract Session

11:30–12:30 Coffee break

11:30–12:30 **Poster Session and e-Poster Presentation**

12:30–14:00 L'Oréal-UNESCO For Women in Science Gala

14:00–14:30 Best Abstract and e-Poster Presentation Awards

14:30–15:30 Lunch break

15:30–17:00 **Plenary Session – RESISTANCE**

15:30–15:50 How integrons helped Gram-negative bacteria to overcome the antibiotic threat – *Didier Mazel*

15:50–16:10 On the emergence and drug resistance of *Mycobacterium tuberculosis* – *Philip Supply*

16:10–16:30 Spread of carbapenemase-producing Enterobacterales in Poland before and during the COVID-19 pandemic – *Marek Gniadkowski*

16:30–16:50 The interplay between climate change, drug resistance and new infectious diseases – *Arturo Casadevall*

16:50–17:00 **Silver Sponsor Forum**

- Challenging AMR with antibiotic stewardship – *Ernesto Battinelli*

17:00–18:00 **Plenary Session – MISCELLANEA**

17:00–17:20 Genetic variability, genotyping, and genomics of *Mycobacterium leprae* – *Philip Suffys*

17:20–17:40 Modern Koch's postulates applied to bacterial pathogenesis of Alzheimer's disease – *Jan Potempa*

17:40–18:00 **Silver Sponsor Forum**

- Microbiological and molecular diagnosis of *Mycobacterium tuberculosis* infections – *Tomasz Szczęsny*

19:00 Reception at the French Embassy in Warsaw – invitation only

Thursday, December 1, 2022

10:00–12:00 Warsaw sightseeing tour – Discovering the history of Warsaw

Farewell and departure of guests

View of Krakowskie Przedmieście, Warsaw, ca. 1884.
Photo by Konrad Brandel, The Museum of Warsaw.

Invited Speakers

Hervé BOURHY

Unit Lyssavirus, epidemiology and neuropathology, Institut Pasteur, Université Paris Cité, Paris, France

Hervé Bourhy is a professor and head of the Global Health Department, the Lyssavirus, Epidemiology and Neuropathology Laboratory, the WHO Collaborating Center for Rabies Reference and Research and the National Reference Center for Rabies at the Institut Pasteur in Paris, France.

His research activities mainly aim to generate a paradigm shift in the way rabies is monitored, studied and controlled, bringing it into the era of One Health and Precision Public Health. His work focuses on questions relating to disease persistence and dispersion, reservoir dynamics (dogs, bats), crossing of the species barrier and modalities and physiopathological consequences of the neurotropism of viruses such as rabies virus and SARS-CoV-2, and development of new antiviral strategies against the rabies virus and SARS-CoV-2. He has published more than 195 papers in peer reviewed international journals.

Roland BROSCH

Unit for Integrated Mycobacterial Pathogenomics, Institut Pasteur, Paris, France

Roland Brosch obtained his PhD at the University of Salzburg, Austria and after his postdoctoral work at the University of Wisconsin and the Institut Pasteur in Paris, he integrated into the scientific staff of the Institut Pasteur.

He worked on groundbreaking genome projects of the tuberculosis agent *Mycobacterium tuberculosis*, the BCG vaccine, and *M. canettii* as well as on the evolution of the *M. tuberculosis* complex, and the discovery and characterization of the ESX / type VII mycobacterial secretion systems.

He is now a professor and head of a research unit and continues to be very interested in the above mentioned topics in order to gain new insights into mycobacterial evolution and host-pathogen interaction, crucial for new vaccine and treatment concepts.

Arturo CASADEVALL
Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

Arturo Casadevall, MD, PhD, is a Bloomberg Distinguished Professor and Alfred and Jill Summer Chair of Molecular Microbiology and Immunology at Johns Hopkins School of Public Health. He received his MD and PhD degrees from New York University. He completed his internship/residency in internal medicine at Bellevue Hospital and specialized in infectious diseases at the Albert Einstein College of Medicine. The author of over 900 papers, books and chapters, his major research interests are in fungal pathogenesis and the mechanisms of antibody action. He is editor-in-chief of mBio, deputy editor of the Journal of Clinical Investigation and serves on several editorial boards. He has served on the National Science Board for Biosecurity and the National Commission on Forensic Science.

He is currently chair of the Board of Governors of the American Academy of Microbiology, the honorific arm of the American Society for Microbiology. He has received numerous honors including election to the American Society for Clinical Investigation, American Academy of Physicians, American Academy of Microbiology, Fellow of the American Academy for the Advancement of Science, American Academy of Arts and Sciences, the National Academy of Medicine and the National Academy of Science.

Stewart COLE

Director of Institut Pasteur, Paris, France

Stewart Cole is an internationally renowned scientist and professor of microbial pathogenesis. Since January 2nd, 2018, he has been President of the Institut Pasteur. From 2007 to 2017, he served as Professor and Director of the Global Health Institute at the Ecole polytechnique fédérale de Lausanne, the Swiss Federal Institute of Technology (EPFL) – a world-leading education and research center.

For 24 years Cole worked as a researcher and also held various research management positions at the Institut Pasteur. He was Director of Strategic Technologies and then Executive Scientific Director, contributing to several patent applications relating to HIV/AIDS, cervical cancer and multidrug-resistant tuberculosis. He participated in the Scientific Advisory Boards of the Institut Pasteur in Iran, the Institut Pasteur in Montevideo and the Institut Pasteur in Lille. Professor Cole was also acting President of the Institut Pasteur in Paris in 2005.

He has been the recipient of many national and international prizes and distinctions. In 2009, he was awarded the World Health Organization's prestigious Stop-TB Partnership Kochon Prize for his leadership and groundbreaking accomplishments in genetic research on *M. tuberculosis* and his contribution to novel therapeutic strategies for tackling TB. During his career, he has been involved in the work of several foundations and scientific committees, and was notably Chair of the Board of the Innovative Medicine for Tuberculosis Foundation and President of the commission médicale for the Fondation Raoul Follereau. Stewart Cole has also published more than 350 scientific papers on infectious diseases, most notably tuberculosis and leprosy. Professor Cole was awarded the title of Chevalier of the Legion of Honor in 2004 and was appointed as Knight Commander of the Order of St. Michael and St. George for service to science, on January 1st, 2022.

Pascale COSSART

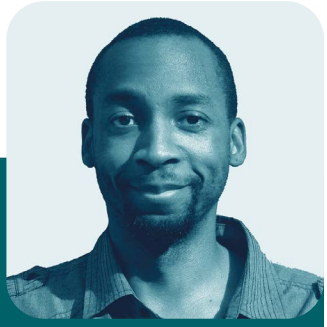
Bacteria-Cell Interactions Unit, Institut Pasteur, Paris, France;
Université de Paris, Paris, France

Pascale Cossart, after studying chemistry in Lille (France), obtained a master's degree at Georgetown University, Washington, DC. Back in France, she obtained her PhD in Paris in the Institut Pasteur, where she had headed the 'Bacteria-Cell Interactions' unit until recently. After studying DNA-protein interactions in *Escherichia coli*, in 1987 she started to study the molecular and cellular basis of infections by intracellular bacteria, taking as a model the bacterial pathogen *Listeria monocytogenes*.

She pioneered the field of Cellular Microbiology. Her research led to new concepts in infection biology, but also in fundamental microbiology – in particular RNA regulation, in cell biology and also in epigenetics. Her contributions have been recognized by many awards, including the Robert Koch Prize (2007), the Louis Jeantet Prize for Medicine (2008), the Balzan Prize (2013), the Heinrich Wieland prize (2018), and the NAS Selman Waksman Award (2021).

She is a member of the French Academy of Sciences (2002), the American National Academy of Science (NAS) (2009), the German Leopoldina (2001), the Royal Society (2010) and the National Academy of Medicine (NAM) (2014).

From 2016 to 2021, she was the Permanent Secretary of the French Academy of Sciences. She is now a scientific visitor at EMBL Heidelberg.



David COUVIN

WHO Supranational TB Reference Laboratory–TB and Mycobacteria Unit, Institut Pasteur de la Guadeloupe, France

Dr. David Couvin is a bioinformatics researcher, working at the Institut Pasteur de la Guadeloupe on various bioinformatics projects, including *Mycobacterium tuberculosis*.

His PhD thesis (which he defended in December 2014, under the supervision of Dr. Nalin Rastogi) focused on the development and refinement of a global database of circulating genotypes of *M. tuberculosis* strains (SITVIT2).

After his PhD thesis, he worked on comparative genomics projects at CIRAD Montpellier (France), then carried out another contract at the Institute of Integrative Biology of the Cell (CNRS, Université Paris-Saclay), which consisted in developing a prediction tool and database of CRISPR-Cas systems from bacteria and archaea genomes.

Patrizia DANESI

Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua, Italy

Patrizia Danesi is a senior researcher at the Laboratory of Parasitology, Mycology and Medical Enthomology at the IZSVe (Istituto Zooprofilattico Sperimentale delle Venezie, Padua, Italy). She graduated in Veterinary Medicine (University of Bologna) and defended her PhD thesis (2014 University of Bari) on epidemiology and genetic characterization of *Cryptococcus* species in feral cats. She obtained the Medical Mycology diploma in the Pasteur Institute (Paris) in 2007.

Her research includes the development of rapid and reliable methods for the diagnosis of fungal and parasitological infection, with a focus on dermatophytes, *Cryptococcus*, *Pneumocystis* and *Prototheca*, *Giardia* and *Leishmania* in companion and wild animals. Together with Professors Malik and Krockenberger (University of Sydney) she is interested in studying the role of animals as sentinels for human exposure to environmental fungal pathogens.

Jean-Marc GHIGO

Genetics of Biofilms Laboratory; Institut Pasteur, Paris, France

Jean-Marc Ghigo runs the Genetics of Biofilms Laboratory in the Department of Microbiology at the Institut Pasteur, Paris. For the past 30 years, he has investigated the molecular bases of various bacterial processes, and since 2001, has been studying new aspects of surface-attached bacterial communities called biofilms.

His research addresses three main inquiries: How do bacteria form biofilms? What properties emerge from bacterial communities? How can we limit biofilm formation?

His laboratory uses bacterial genetics approaches, as well as *in vitro* and *in vivo* models to: i) identify adhesion factors; ii) investigate biofilm-specific properties; iii) study biofilm tolerance to biocides and design anti-biofilm strategies; iv) study bacterial competition within mixed-species communities.

For more information see: Jean-Marc Ghigo - Genetics of Biofilms - Research - Institut Pasteur.

Marek GNIADKOWSKI

Department of Molecular Microbiology, National Medicines Institute, Warsaw, Poland

Marek Gniadkowski received his MSc in 1987 at the Department of Genetics, University of Warsaw, where he then worked until 1995. In 1992 he defended his doctoral dissertation under the supervision of Prof. E. Bartnik. In 1994 he completed a 2.5-year postdoctoral fellowship in the Friedrich-Miescher Institute in Basel, in the laboratory of Prof. W. Filipowicz. In 1995 he started his work in the Central Laboratory of Sera and Vaccines, in the group of Prof. W. Hryniewicz, from 2002 a part of the National Medicines Institute.

Since 1997 he has been the Head of the Department of Molecular Microbiology, dealing with the issues of molecular epidemiology of bacterial infections. His research team focuses mostly on multidrug-resistant Gram-negative bacteria, clonal structure of their populations, and the mechanisms of β-lactam resistance.

In 2004 he defended his habilitation, and in 2011 obtained the title of professor of medical sciences at the Medical University of Warsaw.

Andrzej GÓRSKI

Bacteriophage Laboratory, Department of Phage Therapy, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland; Department of Clinical Immunology, Infant Jesus Hospital, Medical University of Warsaw, Poland

Andrzej Górski is a professor of medicine and immunology at the Medical University of Warsaw. He specializes in internal diseases, immunology, and transplantology.

Since 1999 he has headed the Phage Therapy Unit of the Medical Centre of the L. Hirszfeld Institute of Immunology and Experimental Therapy at the Polish Academy of Sciences. He was Director of this Institute from 1999 to 2007, Vice-Rector (1993–1996), Rector of the Medical University of Warsaw (1996–1999) and Vice-President of the Polish Academy of Sciences (2007–2015), as well as a Member of the European Group on Ethics in Science and New Technologies (2011–2015).

Prof. Górski had several research appointments at various prestigious foreign institutions (e.g. Sloan-Kettering Institute for Cancer Research, USA; Fred Hutchinson Cancer Research Center, USA; Karolinska Institutet, Sweden; Weizmann Institute of Science, Israel) and received many scientific awards, including the Meller Award for excellence in cancer research from the Sloan-Kettering Institute, the ICRETT Award and the Yamagiwa-Yoshida Award from the International Union Against Cancer, the Jędrzej Śniadecki Memorial Award from the Polish Academy of Sciences.

Prof. Górski has authored more than 430 scientific publications (H-index 45). He serves as the editor-in-chief of *Archivum Immunologiae et Therapiae Experimentalis*, and as an editor of Science & Engineering Ethics.

Sybren DE HOOG

Radboud University Medical Center, Nijmegen, The Netherlands

Sybren de Hoog is a senior researcher at the Center of Expertise in Mycology of Radboud University Medical Center and Canisius Wilhelmina Hospital in Nijmegen, The Netherlands.

He was a professor of mycology at the Institute of Biodiversity and Ecosystem Dynamics of the University of Amsterdam, and held visiting professorships at universities of Beijing, Guiyang, Nanjing and Suzhou in China, and Curitiba in Brazil. He is the past-President of the International Society for Human and Animal Mycology (ISHAM). In this function he assisted in the organization of ISHAM and satellite congress in Tokyo and Beijing in 2009. He was the program chairman of the TIFI/ECMM congress in Amsterdam (2003) and of the ISHAM Congress in Amsterdam (2018).

He is the first author of the Atlas of Clinical Fungi, which appeared in print in 2020 (1599 pages, >4000 citations) and for which there is a continually updated electronic version with molecular data available.

His teaching activities comprise the International Course Medical Mycology for hospital personnel, with editions in Europe, China, USA and Brazil. Currently he (co-)guides seven international PhD students and postdocs.

His research focuses on evolution of medically relevant fungi in dermatophytes and black yeasts. He has written more than 900 scientific publications (H-index 107).

Łukasz KOZUBOWSKI

Department of Genetics & Biochemistry, Eukaryotic Pathogens Innovation Center, Clemson University, Clemson, South Carolina, USA

Dr. Łukasz Kozubowski received his MS degree in pharmaceutical sciences at the Medical University of Warsaw under the mentorship of Dr. Józef Sawicki and Dr. Grzegorz Nałęcz-Jawecki; and a doctorate in Biochemistry and Molecular Biology at the Louisiana State University Medical Center under the mentorship of Dr. Kelly Tatchell.

He then conducted postdoctoral studies on establishment of cell polarity based on *S. cerevisiae* model in the laboratory of Dr. Daniel Lew at the Duke University, followed by studies on pathogenesis of *Cryptococcus neoformans* in laboratories of Dr. Joseph Heitman, Dr. Andy Alspaugh, and Dr. John Perfect.

He is an Associate Professor at Clemson University, where his research group investigates the impact of stress response on fungal pathogenesis and susceptibility to antifungal drugs.

Marc LECUIT
Département de Biologie Cellulaire et Infection, Institut Pasteur, Paris, France

Marc Lecuit is a microbiologist and an infectious diseases physician. He is the Director of the Biology of Infection Unit at the Institut Pasteur and Inserm, and the Chair of the Department of Cell Biology and Infection at the Institut Pasteur.

Marc Lecuit is a professor at Université Paris Cité and senior attending physician, Deputy Head of the Department of Infectious Diseases and Tropical Medicine at the Necker-Enfants Malades University Hospital. His research focuses on understanding molecular mechanisms underlying the ability of microbes to target specific hosts, infect host cells, cross host barriers and disseminate systemically and within tissues, as well as on how host responses affect infection outcome. His laboratory focuses on pathogens that have the ability to induce maternal-fetal and central nervous system infections.

Marc Lecuit has made important contributions to the understanding of the biology of infections caused by *Listeria monocytogenes*, as well as emerging pathogens such chikungunya, Zika and SARS-CoV-2 viruses. He is also involved in translational research projects in the Institut Pasteur international network.

Marc Lecuit is supported by the European Research Council. He is an appointed Fellow of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), a member of the European Molecular Biology Organization (EMBO) and Academia Europaea and senior member of Institut Universitaire de France.

Jean-Claude MANUGUERRA
Unité Environnement et Risques Infectieux, Institut Pasteur, Cellule d'Intervention Biologique d'Urgence (CIBU), Paris, France

Jean-Claude Manuguerra (JCM) was originally qualified as a veterinarian at the Ecole Nationale Vétérinaire d'Alfort close to Paris, and was trained in virology at the Institut Pasteur in order to get his PhD at the University Paris XI. He spent two years as a post-doctoral fellow at the National Institute for Medical Research (London, United Kingdom). He was then a co-director of the National Influenza Centre for Northern France, one of the 13 laboratories included in the WHO Collaborative Multi-Centre Laboratory Network on SARS (1994–2003). He belonged to the French team sent to Hanoi for the control of the SARS outbreak in March 2003. Since then, he participated in a number of missions during outbreaks (seasonal influenza, Madagascar 2002; SARS Hanoi 2003; H5N1 avian influenza, Phom Pehn 2004; pandemic influenza H1N1, Mexico 2009; MERS, Riyad 2013; Ebola, Conakry 2014 & Macenta 2015).

From 2000 to 2018, JCM was a member of the steering committee of the Global Alert and Response Network (GOARN) and chaired it from 2011 to 2013. From 1998 to 2001, he was the Secretary General of the French Society for Microbiology. He was a member of the Scientific Council of the National Veterinary School of Alfort (2005–2014). From 2003 to 2010, JCM was the Chair of the National French Committee for influenza pandemic planning.

Currently, JCM – Research Director at the Institut Pasteur, heads the Environment and Infectious Risks expertise and a research unit which harbors the Laboratory for Emergency Response to Biological Threats and the National Reference Centre for Hantaviruses. He has been the Chief Editor of Intervirology since 2012.

JCM is currently the Vice-Chairperson of SAGO (WHO Scientific Advisory Group on the Origin of emerging and re-emerging pathogens with pandemic and epidemic potential).

Since 2005, he has been a corresponding member of the Académie Vétérinaire de France, which Louis Pasteur was a member of. JCM holds the titles of Chevalier de l'Ordre National du Mérite (Knight of the National Order of Merit) and Chevalier de la Légion d'Honneur (Knight of the Legion of Honour) – which are prestigious French honors.

Robin MAY
Institute of Microbiology & Infection, School of Biosciences,
University of Birmingham, Birmingham, United Kingdom

Robin May is a Professor of Infectious Diseases within the Institute of Microbiology & Infection at the University of Birmingham, UK. He is currently on a 60% secondment to the UK Government, serving as Chief Scientific Adviser to the Food Standards Agency.

His early training was in Plant Sciences (University of Oxford), followed by a PhD on mammalian cell biology with Prof. Laura Machesky (University College London & University of Birmingham). From 2001–2004 he was a Human Frontier Science Program fellow with Prof. Ronald Plasterk at the University of Utrecht, The Netherlands, working on RNA interference mechanisms. In 2005 he obtained a Research Council UK Fellowship to establish his own group at the University of Birmingham. In 2010 he was awarded a Lister Fellowship, in 2013 he was presented with the Colworth Medal of the Biochemical Society and in 2015 he received Wolfson Research Merit Award from the Royal Society. From 2014–2020 Prof. May held a Consolidator Award from the European Research Council and in 2020 was elected to Fellowship of the American Academy of Microbiology.

Prof. May's research interests focus on host-pathogen interactions and, in particular in understanding how some pathogens are able to subvert the innate immune system. Much of his work is aimed at improving the treatment or prevention of opportunistic infections in patients with impaired immunity, such as HIV-positive individuals, patients in critical care, or people with long-term immune-compromising conditions.

Didier MAZEL

Department of Genomes & Genetics, Institut Pasteur, Paris, France

Didier Mazel in 1990 defended his PhD in Molecular and Cellular Genetics on the characterization of the genes encoding the light harvesting complexes in cyanobacteria. In parallel, he demonstrated that metabolic constraints linked to elemental sulfur availability in the different types of water colonized by cyanobacteria, were imprinted in their protein sequences.

Since then, he has made several important contributions to the study of antibiotics and antibiotic resistance, including understanding of the mechanisms of the integron capture and dissemination of resistance genes.

Didier Mazel and his collaborators study genome organization and chromosome maintenance mechanisms in *Vibrio cholerae*, which is constituted of 2 circular chromosomes (chr). They identified the unique mechanism that coordinates chr2 replication, to ensure synchronous replication termination for the two chromosomes.

Recently, he became one of the pioneers who leverage synthetic biology to invent novel antimicrobials by developing an original system that kills specifically *V. cholerae* in complex populations.

Jan POTEMPA

Department of Microbiology Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland;
Department of Oral Health and Systemic Disease, University of Louisville School of Dentistry, University of Louisville, Kentucky, USA

Jan Potempa defended his PhD (1982) and DSc (habilitation, 1993) in Biochemistry at the Jagiellonian University, Krakow, Poland. He holds a Doctorate Honoris Causa from the University of Lund and University of Amsterdam.

Currently, Dr. Potempa is affiliated with the University of Louisville Dental School, where he is a Full Professor and Distinguished Academic Scholar. He also maintains a teaching and research position at the Jagiellonian University, where he is a Research Professor (since 2005) and Head of the Department of Microbiology (since 2001).

He received the most prestigious awards for scientific achievements in Poland: FNP Prize (2011) and the Heisig Award (2021).

His current investigations are focused on virulence factors of bacterial pathogens that play important roles in the dysregulation of several physiological pathways and evasion of host immunity, especially in the context of periodontitis and associated diseases.

Daniel RAICHVARG

University of Burgundy / EPCC Terre de Louis Pasteur, Dijon, France

Daniel Raichvarg obtained his PhD at the University of Paris 7, France, in the fields of communication, pedagogy, and history of sciences. He held a position of senior lecturer at the University of Paris-Orsay in these fields. He then became a full professor of communication sciences at the University of Burgundy, where he created a research unit in communication sciences (Lab. CIMEOS) and developed the Science and Society programme at the same university. He is now professor emeritus of communication sciences at the University of Burgundy. He is a Honorary President of the French Society for Information and Communication Studies, after a two-term Presidency.

He is an international expert in the fields of science and society-related issues and cultural outreaches. He is helping to develop projects of Terre de Louis Pasteur – sciences and heritage, public cultural cooperation establishment under the responsibility of the French Academy of Sciences.

Juan-Luis RAMOS

Estación Experimental del Zaidín, Consejo Superior
de Investigaciones Científicas, Granada, Spain

Juan L. Ramos is a Full Professor at CSIC-EEZ in Granada. His multidisciplinary research approach comes from the “bench to field” process, integrating molecular biology, chemical engineering and field assays. He has greatly deepened the knowledge of microbial physiology, genetics and molecular ecology and applied it in order to come up with better ways of restoring polluted sites using microbial biodegradation.

Juan L. Ramos has supervised 47 PhD theses and the work of 50 post-docs from 5 continents. He is an elected member of the American Academy of Microbiology, the European Academy of Microbiology and Académico Numerario (medal 13) of the Granada Academy of Sciences. In 2012 he received the prestigious Jaime I Award for Environmental Protection, and in 2013 – the Lwoff medal of FEMS in recognition for his contribution.

Uwe RÖSLER

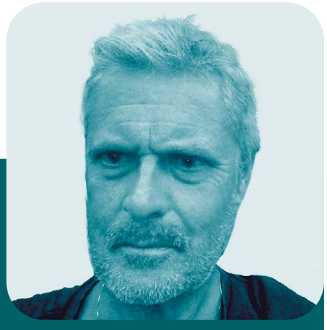
Institute for Animal Hygiene and Environmental Health, Centre for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Germany

Uwe Harry Rösler was born in 1971, is married and has two almost grown-up sons.
He studied veterinary medicine in Leipzig, Germany, until 1997. He received his doctorate degree in 2001 and his habilitation in 2007, both at the Institute for Animal Hygiene and Veterinary Public Health at the University of Leipzig. He received appointments to senior professorships (W3) in animal and environmental hygiene in i.a. Stuttgart (Hohenheim), Hanover and Berlin.
He has been a Senior Professor for Animal Hygiene and Infectiology at the Department of Veterinary Medicine at the Free University of Berlin since 2008, where he holds the position of Head of the Institute for Animal Hygiene and Environmental Health. He is currently the Dean of the Department of Veterinary Medicine at the Free University of Berlin and is also involved in numerous national and international committees focused on infection prevention and disinfection.
His research interests focus on the epidemiology and control of infectious diseases (including *Salmonella*, *Campylobacter* and *Prototheca* infections) and antimicrobial resistance.

Ilan SCHWARTZ

The Department of Medicine, Duke University School of Medicine, Durham, North Carolina, USA

Ilan Schwartz is an infectious diseases physician and researcher in the Division of Infectious Diseases at Duke University.
His clinical and research interests involve emerging fungal infections, immunocompromised hosts, and global health. After his clinical training in Canada, he obtained a Doctorate in Medical Sciences from the University of Antwerp for research on emergomycosis, a novel opportunistic fungal infection affecting people with advanced HIV in South Africa, followed by a research fellowship at the San Antonio Center for Medical Mycology.
He spent 4 years as a transplant ID physician and researcher at the University of Alberta in Canada before being recruited to the Duke Mycology Research Unit in August 2022.



Philip SUFFYS

Laboratório de Biologia Molecular Aplicada a Micobactérias,
Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil

Philip Suffys graduated in biology from the State University of Gent, Belgium (1985). He received his PhD degree in molecular biology at the same university (1989).
In 1990, he started working at the Oswaldo Cruz Institute at Fiocruz (Rio de Janeiro, Brazil), which belongs to the Pasteur Network. There, he heads the Laboratory of Molecular Biology Applied to *Mycobacteria*.
Dr. Suffys has strong expertise in genetics and microbiology with special emphasis on mycobacteria-related issues, including molecular typing, genomics, phylogeny, and drug resistance in tuberculosis, leprosy and mycobacteriosis.
Recently, he has also been active in *Legionella* infections and syphilis. Between 2015 and 2016, he completed a post-doc at the Institute of Tropical Medicine in Antwerp. He is an author of more than 150 research papers in peer-reviewed journals and his h-index exceeds 30.

Philip SUPPLY

National Center for Scientific Research (CNRS), Lille, France;
Centre for Infection and Immunity, Institut Pasteur de Lille, France

Philip Supply is a Research Director of the National Center for Scientific Research at the Institut Pasteur de Lille, France.
He dedicates his research to the genomics and evolution of *Mycobacterium tuberculosis*. Based on the discovery of evolutionarily early branching lineages of Tuberculosis bacilli, his work provided unique insights into the origin and emergence of the pathogen.
He developed the 24-locus MIRU-VNTR genotyping approach adopted as the worldwide standard for epidemiological tracing and surveillance in the pre-genomic era.
Together with GenoScreen, he designed and developed the Deeplex®-MycTB kit, representing the most comprehensive test available for culture-free diagnosis of drug-resistant tuberculosis based on targeted next generation sequencing.

Michel TIBAYRENC
Maladies Infectieuses et Vecteurs Ecologie, Génétique, Evolution et Contrôle, Institut de Recherche pour le Développement, Montpellier, France

Michel Tibayrenc has worked on the genetics and evolution of infectious diseases for more than 35 years.

He is a director emeritus of research at the French Institut de Recherche pour le Développement (IRD), founder and editor-in-chief emeritus of Infection, Genetics and Evolution (Elsevier), and founder and principal organizer of the international congresses MEEGID (molecular epidemiology and evolutionary genetics of infectious diseases), supported and managed by Elsevier.

He is the author of more than 200 international papers (H index = 49). He has published 6 scientific books: (1) American trypanosomiasis: Chagas disease. 100 years of discovery (Elsevier; first editor: Jenny Telleria; 2010; reedited 2017); (2) Genetics and evolution of infectious diseases (Elsevier; 2010; reedited 2017; 3rd edition planned 2022); (3) On Human Nature (Elsevier/Academic press; 2016; coeditor: Francisco J. Ayala); (4) What makes us humans (2020; Nova science publishing; coeditor: Francisco J. Ayala).; (5) Spanish edition: Lo que nos hace humanos (SAL TERRAE; coauthor: Francisco J. Ayala); (6) French edition: Notre humaine nature (éditions rue de Seine, Paris; in press; coauthor: Francisco J. Ayala).

Together with his collaborator – Jenny Telleria, he is the founder and scientific advisor of the Bolivian Society of Human Genetics (2012).

Violeta VALCHEVA

The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

Violeta Valcheva is the Head of Laboratory of Molecular Biology of Mycobacteria in the Department of Infectious Microbiology, at the Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria. She is an Associate Professor at the The Stephan Angeloff Institute of Microbiology (SAIM), Bulgarian Academy of Sciences, Sofia, Bulgaria.

In 2009 she received her PhD and conducted the first comprehensive study of molecular characterization and drug resistance of *Mycobacterium tuberculosis* strains in Bulgaria. The main research topics of her work are the molecular epidemiology, phylogeny and evolution of mycobacteria, bioinformatics, bacterial pathogenesis and virulence; new synthesized compounds with chemical and natural products, drug development, pharmacokinetics, anti-TB chemotherapy.

She gained experience and high qualifications at various international institutions (in France, Russia, China, Japan) with prominent results (projects, publications, establishing new innovations and collaborations). She is responsible for public relations at SAIM, and for SAIM's scientific relation in the International Pasteur Network. She was a secretary of the Microbiology Department in the Bulgarian Union of Scientists in the period of 2009–2013.

José A. VÁZQUEZ-BOLAND

Microbial Pathogenesis Laboratory, Infection Medicine, Edinburgh Medical School (Biomedical Sciences), University of Edinburgh, Edinburgh, United Kingdom

José Vázquez-Boland is a Professor at the University of Edinburgh's Medical School where he holds the chair of infectious diseases. He has a veterinary degree from Complutense University of Madrid (1985), a diploma in public health (1986), and spent time in clinical practice and as public health officer before earning his PhD degree (1990). After postdoctoral studies at the Institut Pasteur (1990–1991), back in Spain he established a research group in microbial pathogenesis. A University Professor since 1993, in 2002 he took up a chair of molecular microbiology at the University of Bristol before moving to Scotland in 2007. His research interests lie primarily in the virulence, adaptive mechanisms and genomics of facultative pathogens with a focus on *Listeria* and *Rhodococcus equi*. He received the Spanish Society for Microbiology "Jaime Ferran" biennial award, the EU Descartes Prize for Transnational Cooperative Research, and is an elected Fellow of the American Academy of Microbiology.

Miguel VIVEIROS

Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Portugal

Migule Viveiros is a Professor in Biomedical Sciences – Medical Microbiology and Global Health, Vice-Director of the Institute of Hygiene and Tropical Medicine, NOVA University of Lisbon, Portugal. He is devoted to the early diagnosis and molecular epidemiology of TB and new therapeutics for MDRTB and XDRTB.

He is a specialist in the diagnosis of antibiotic resistance in bacteria by phenotypic and genotypic assays; executive committee member of the Study Group for Mycobacterial Infections of ESCMID; Scientific Coordinator of 'Ciência LP' – Center for Advanced Training in Fundamental Sciences for Scientists from Portuguese-speaking Countries (UNESCO Category 2 Center) and Vice-President of the Portuguese Society for Microbiology.

He is the author/co-author of over 200 scientific papers in peer reviewed journals and books in the field of mycobacteriology, resistance to antibiotics and microbial genetics.

For more information see:

- Miguel Viveiros — Universidade NOVA de Lisboa (unl.pt)
- Miguel Viveiros (0000-0001-9676-6251) (orcid.org)

Michał WANDEL

Laboratory of Intracellular Immunity, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Michal Wandel is an EMBO Installation Grantee and a leader of the Laboratory of Intracellular Immunity (at the IBB PAS, Warsaw, Poland), who employs a combination of molecular biology, cell biology, biochemistry and microbiology to investigate intracellular anti-microbial defense mechanisms of innate immunity.

He obtained his PhD at the University of Cambridge and then significantly contributed to the understanding of host-pathogen interactions during his postdoctoral research at the MRC Laboratory of Molecular Biology in Cambridge, where he investigated how interferon-enhanced immunity protects the host cells against bacterial invasion and discovered the GBP-CASP4 signaling platform.

Daniel WIBBERG
Centrum für Biotechnologie, CeBiTec, Universität Bielefeld, Germany;
Institute of Bio- and Geosciences IBG-5, Computational Metagenomics,
Forschungszentrum Jülich GmbH, Jülich, Germany

Dr. Daniel Wibberg is a bioinformatician, data manager and trainer with more than ten years of experience working with all kinds of sequencing data. He holds a B.Sc. in Bioinformatics & Genome Research and a Master's Degree in Genome based Systems Biology both from Bielefeld University. In his PhD thesis, he analyzed the genome and transcriptome of the pathogenic fungus *Rhizoctonia solani* AG1-IB.
He specializes in genomics, transcriptomics and metagenomics and was involved in more than 250 different projects. Since 2015, he has been working as training coordinator and trainer in the German Network for Bioinformatics Infrastructure (de.NBI) representing the German ELIXIR node. From 2022, the Forschungszentrum Jülich GmbH as a member of the Helmholtz Association of German Research Centers has been entrusted with the consolidation of the de.NBI / ELIXIR Germany. Therefore, Daniel Wibberg is member of ELIXIR Germany administration office of Institute of Bio- and Geosciences (IBG) 5 "Computational metagenomics" at FZ Jülich.

Main Avenue in the Saxon Garden; in the background, the colonnade of the Saxon Palace, Warsaw, 1890s.

Photo by Konrad Brandel, The Museum of Warsaw.

Special Guests

Judith ARMITAGE

The Lister Institute; University of Oxford, United Kingdom

Judith Armitage FRS is a Professor of Bacterial Biochemistry at University of Oxford. Her research has centred on bacterial responses to changes in their local environment, specifically how they control swimming to move to favourable environments, combining molecular genetics and biochemistry with biophysics, single molecule live cell imaging and modelling. She is a Fellow of The Royal Society, American Society of Microbiology, European Society of Microbiology and a member of EMBO. She was President of the Microbiology Society from 2019 to 2022. Her independent career was enabled by a Lister Institute Fellowship and she has been a member of their Governing Body since 2015.

In addition to being a Fellow of the Lister Institute, Judith Armitage is a Fellow of The Royal Society, American Society of Microbiology, European Society of Microbiology and a member of EMBO. She was President of the Microbiology Society from 2019–2022.

Hilary LAPPIN-SCOTT

FEMS President; Cardiff University, United Kingdom

Hilary-Lappin Scott is a Professor of Microbiology and has had an extensive career as a research scientist at Exeter University for 20 years, prior to moving into senior University leadership roles. She has been appointed Pro-Vice-Chancellor for research and innovation at Bangor University before moving to Swansea University in 2010. She is the third female President of FEMS, and the first from the UK and Ireland. She is a renowned microbiologist, who has spent the majority of her career researching microbial biofilm communities. Hilary Lappin-Scott has also been an active ambassador for science and microbiology, and is an avid supporter of advancing science for people of all identities and backgrounds. She is a former President of the Microbiology Society, one of our Member Societies and is a previous President of ISME.

Agata BUDKOWSKA
Former Chief of Laboratory at the Pasteur Institute
and Scientific Advisor for the Department of International Affairs, Paris, France

After completing an MSc in Biological Sciences at the University of Warsaw followed by a PhD, she was in charge of the Immunochemistry Laboratory at the National Institute of Public Health in Warsaw. She then moved to the USA to work at the National Institutes of Health (NIH) in Bethesda with an International Fogarty Centre Fellowship. She joined the Pasteur Institute in Paris in 1985, where she for 30 years headed research on Hepatitis B (HBV) and Hepatitis C (HCV) viruses.

She was first to elucidate the structure and properties of HBV nucleocapsids, the role of the HBV envelope Pre-S proteins and corresponding antibodies in HBV infection and for vaccination. Next, she investigated HBV cell entry mechanisms and the role of hepatic receptors in the initiation of the HBV infection.

She discovered non-enveloped HCV nucleocapsids circulating in the blood of infected patients, and described their structure and properties. She initiated studies and demonstrated the essential role of lipoproteins in the formation of HCV virions, in the HCV cell entry via interaction with hepatic lipoprotein receptors and their role in the virus escape mechanisms from the host immune response.

Following her successful career as a Chief of Laboratory at the Pasteur Institute in Paris, she continued her involvement with the Institute as a Scientific Advisor for the Department of International Affairs.

She was made Professor of Medical Sciences by the President of Poland in 2014.

Waleria HRYNIEWICZ

Department of Epidemiology and Clinical Microbiology,
National Medicines Institute, Warsaw, Poland

Waleria Hryniewicz received her medical degree from the Medical University of Warsaw. She was the recipient of the Wellcome Trust grant for research on the biological properties of L forms of *Streptococcus pyogenes*, which was also the topic of her PhD thesis at the Medical University of Warsaw. She held the position of the Head of Staphylococcal and Streptococcal Laboratory focused on purification and activities of toxins. She was a post doctorate fellow at the University of Minnesota , working on the biological properties of streptolysin S and after her return to Poland, she was appointed as a full professor and the scientific director of the National Institute of Hygiene. Finally, she moved to Sera and Vaccine Central Laboratory (currently a part of the National Medicines Institute) where, as the managing director, she initiated research on molecular epidemiology of invasive bacterial infections, in particular on pneumococci and meningococci and the epidemiology and mechanism of antibiotic resistance of major human pathogens with special emphasis on Gram-positive cocci. She was the Chair of Microbiology Committee of the Polish Academy of Sciences and the President of the Polish Society of Microbiologists. She also served as the national consultant to the Ministry of Health for clinical microbiology. She is an author of over 350 publications, mostly in international peer review journals. She is an ESCMID fellow and is on the Stanford list of 2% of the most cited international scientists.

Grzegorz WĘGRZYN

Department of Molecular Biology, Faculty of Biology,
University of Gdańsk, Poland

Grzegorz Wegrzyn obtained his PhD degree in 1991 at University of Gdańsk (Poland), after which he was a research fellow at the University of Nottingham (UK), and a post-doctoral researcher at the University of California, San Diego (USA). Since 1996 he has been a Professor and Head of the Department of Molecular Biology at the University of Gdańsk (Poland). His research is focused on regulation of gene expression and DNA replication, control of development of bacteriophages, and mechanisms and new treatment methods of human genetic and neurodegenerative diseases. He has supervised 54 PhD theses, led over 30 research projects, and published over 400 scientific articles. He is an editor in several scientific journals.

Plenary Session (O)

Plenary Session – Prelude

O001 The Institut Pasteur today and the legacy of Louis Pasteur

Prof. Sir Stewart Cole[1]

[1] President of the Institut Pasteur

December 27th, 1822 is the birthdate of Louis Pasteur, a global figure in scientific and medical research. Pasteur excelled in four different areas of science: chemistry, biotechnology, microbiology and infectious diseases and, especially, vaccine development. In 1885, his rabies vaccine met with universal acclaim.

The Institut Pasteur, a private foundation with officially recognized charitable status established by Louis Pasteur in 1887, is an internationally renowned center for biomedical research and the hub of the Pasteur Network, comprising 33 institutions worldwide. The institute operates in four main areas: scientific and biomedical research, public health and surveillance, teaching and training, and technology transfer. More than 2,800 people work on its Paris campus and 10 Pasteur scientists have been awarded the Nobel Prize for Medicine, the most recent being for discovery of the HIV/AIDS virus.

The "Pasteur ethos" is rooted in strong scientific and humanitarian values and fosters three key ambitions: understanding the living world, improving human health, and sharing knowledge. Two centuries later, the "Pasteur ethos" continues to inspire the world.

Over the years, the Institut Pasteur has evolved to embrace new areas of science and has diversified its research portfolio while retaining a strong commitment to microbiology, infectious diseases and immunology. In this presentation I will give three examples of some recent major achievements in Covid-19 research, vaccinology and neurosciences, all of which reflect the Pasteur ethos.

Louis Pasteur, a child of the Jura, a man for the world

O002

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[1] University of Burgundy / EPCC Terre de Louis Pasteur, Dijon, France

How did Louis Pasteur, born in a small town in the Jura, still little known to the world today (Dole), become a man known throughout the world? We would like to try to bring two series of elements to answer this question.

The first series of elements concerns Pasteur's relationship to the representation of reality. Acquired in stages from the age of 14 through his practice of various artistic forms, this interest gradually became "scientificized" at the same time as new forms of reproduction of reality such as photography, appeared.

The second series of elements concerns the involvement of Pasteur's research in production practices based on nature, which we could call "his relations with his fields". Here again, inscribed in his youth and his territory made of leather and vines, this interest progressively multiplies his method because the scientific objects treated concern objects which, themselves, induce processes of communication not only scientific but with numerous social actors (ferments, silkworm diseases).

Throughout his work, Pasteur had to provide himself with the means to set up this interdisciplinarity and communication, both of which took very different forms, and constituted his method. The result was his great work: the Pasteur Institute, or rather the Pasteur Institutes and the Pasteur network.

Plenary Session – P-athogens

O003 How the food borne pathogen *Listeria monocytogenes* promoted the emerging discipline "Cellular Microbiology"

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In the late eighties, combined efforts in genetics, molecular biology, and cell biology allowed to investigate how both the pathogen and the host target cell react during an infection. A new discipline that we named "Cellular Microbiology" emerged which provided gargantuan advances in our knowledge of the mechanisms used by both the pathogens and the target cells during their interactions.
Several Gram-negative bacteria (e.g. *Salmonella*, *Shigella*, *Yersinia*) were dissected in detail. We focused our efforts on the Gram-positive food borne pathogen *Listeria monocytogenes*, a bacterium responsible for gastroenteritis, meningitis, materno-fetal infections leading to abortions or premature deliveries with dramatic neurological sequelae. *Listeria* infections are lethal in 30% of the cases which mainly occur among immuno-compromised individuals, elderly or pregnant women.
Our laboratory contributed to several discoveries which illustrate how the study of a single bacterial species can reveal general principles in infection biology and bring important contributions in cell biology as well as fundamental microbiology. Some will be presented during the talk.

O004 Outsmarting the host: *Listeria* interplay with host cells and tissues

Marc Lecuit[1]

[1] Institut Pasteur, Inserm, Université Paris Cité, Necker-Enfants Malades Hospital Paris, Paris, France

Deciphering the molecular mechanisms underlying the targeting and invasion of host cells by microbes, and their dissemination within host tissues is a way forward to understand host biology better and improve health. We study the molecular mechanisms by which microbes cross mucosal barriers, disseminate systemically, and cross within-host barriers such as the blood-brain and placental barriers.
We focus on the model microorganism *Listeria monocytogenes* (*Lm*), a bacterial foodborne pathogen which causes listeriosis, a severe and often fatal human infection. Once ingested, *Lm* is able to actively cross the intestinal, placental and blood-brain barriers leading to septicemia, fetal and central nervous system infection, respectively. We will present the mechanisms by which *Lm* crosses these barriers and highlight how *Lm* ability to circumvent host defenses is selected for, as it promotes its survival and dissemination within the host and its release into the environment.

Control of the saprophyte-to-pathogen transition in *Listeria*: mechanism and clinical implications

O005

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Listeria monocytogenes virulence is controlled by PrfA, an allosterically regulated transcription factor. PrfA is essential for both coordinating the activation of the listerial virulence programme during infection and preventing wasteful production of virulence factors outside the host, thus maximizing the pathogen's transmission fitness. I will review how *L. monocytogenes* PrfA senses the transition from saprophyte to pathogen via the peptide composition of the bacterial habitat and discuss new data about the structural mechanism of promiscuous PrfA inhibition by nutritional peptides. The presentation will also illustrate how basic research into PrfA regulation and the mechanisms of *Listeria* intracellular parasitism can translate to direct clinical applications in the treatment of listeriosis.

Interplay between stress and drug resistance in pathogenicity of *Cryptococcus neoformans*

O006

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Cryptococcus neoformans is a pathogenic yeast-like fungus that causes lethal meningitis in immunocompromised patients. One of the challenges in treating cryptococcosis, especially in the geographical areas where the infection is most prevalent, is the development of resistance to azole antifungals. Previous studies have demonstrated that resistance to the azole drug fluconazole (FLC) results from elevated copies of critical resistance genes in aneuploid cells. However, how aneuploidy is formed in the presence of FLC remains unclear. Our studies explore the effects of FLC on *C. neoformans* at a cellular and population level. We find that FLC leads to pleiotropic defects, affecting synchronization between growth and cell cycle progression. Our data also suggest that FLC causes DNA damage through forming complexes with metals and generating reactive oxygen species, which may contribute to development of resistance. At a population level, we characterize factors that influence phenotypic heterogeneity within *C. neoformans* population and how they affect the response to FLC. We model how variable reaction to FLC within the population changes depending on FLC concentration and factors that influence the rate of cellular growth. Our findings point to a multifactorial nature of the evolution of the resistance to FLC.

O007 New developments in rabies post-exposure prophylaxis and therapy

Hervé Bourhy[1]

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Rabies is a severe viral infection that causes an acute encephalomyelitis, which presents a case fatality of nearly 100% after the manifestation of neurological clinical signs. Rabies can be efficiently prevented with post-exposure prophylaxis (PEP), composed of vaccines and anti-rabies immunoglobulins (RIGs). However, the PEP protocol faces access and implementation obstacles in resource-limited settings, which could be partially overcome by substituting RIGs for monoclonal antibodies (mAbs). Further, no treatment exists for symptomatic rabies. We will show how we have been working on the development of new approaches and strategies to identify effective human monoclonal antibodies neutralizing the rabies virus, how their mode of action was characterized and how, for the first time, some of them proved to be efficient in a therapeutic model of rabies.

O008 Immune evasion by a lethal fungal pathogen

Robin May[1]

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My group are interested in host-pathogen interactions and, in particular, in understanding how some pathogens are able to subvert the innate immune system. Most of our work focuses on phagocytic cells, which some microorganisms are able to use as a “safe house” within which to replicate. We try and understand how such pathogens can survive inside this hostile environment and the effect this intracellular reservoir has on disease progression.

The major focus of our group is on eukaryotic infections, with a particular interest in the lethal fungal disease cryptococcosis. In this talk I will discuss our recent work revealing two intriguing ways in which Cryptococci manipulate the host to achieve long-term persistence. Firstly, we will discuss the phenomenon of Titan cell formation, in which fungal cells enlarge enormously within the lung to avoid phagocytosis and drive dissemination. Initially discovered in *Cryptococcus neoformans*, our recent work has highlighted some important differences in this process within the other main pathogenic species *C. gattii*, which may help to explain clinical differences between the two pathogens.

Secondly, we will show some recent data revealing how Cryptococci produce a potent inhibitor of antigen presentation that dampens T-cell responses and permit fungal persistence in the absence of inflammation. Understanding more about this molecular process may hold promise not only for combatting this life-threatening infection but perhaps also in highlighting novel strategies for therapeutic anti-inflammatory development.

Plenary Session – A-nimals

The challenge of antimicrobial resistances on the animal-human-environment interface

O009

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Animals as sentinels for environmental fungal pathogens

O010

Patrizia Danesi[1]

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Cryptococcosis is a sporadic but uncommon fungal infection encountered throughout the world. It is more prevalent in certain geographical areas, e.g. Australia, the Pacific North West of North America. The range of animals susceptible to infection is perhaps greater than for any other pathogen. It includes a wide diversity of wildlife, companion and production animals, and people. As well as in koalas and companion animals, clinical infections and/or asymptomatic carriage are reported in many terrestrial and aquatic placental mammals, marsupials, monotremes, birds, reptiles, amphibians, fish and amoeba. The *Cryptococcus neoformans* and *C. gattii* species complexes have been identified as the main causes of infection in wildlife, with higher prevalence in animals from endemic areas (Western Canada, Australia and Brazil, respectively), and especially in koalas. In general, results from such studies suggest that wild animals are more likely to be asymptomatic carriers (*i.e.* subclinical disease) than clinically affected by cryptococcosis. Overall, migratory birds show lower prevalence of *C. neoformans/C. gattii* isolates compared to less virulent species such as *C. albidus* and *Papiliotrema laurentii* (previously *Cryptococcus laurentii*). We contend that surveillance of wildlife can provide an early warning system for outbreaks of new or emerging variant pathogen strains, helping to undertake an accurate risk assessment. Standardization of protocols and tools is needed to make a global comparison possible. In other way, wildlife species can be very important sentinels for the *Cryptococcus neoformans/gattii* species complexes and provide new and penetrating insights into the epidemiology and pathogenesis of disease caused by these organisms.

Gold Sponsor Forum

O011 Supporting your microbes - how Th. Geyer offer can make your lab get fit for growth

Paweł Stępniak

The ever-growing disciplines of life sciences require from your laboratory workflow engaging new techniques and methodologies every day. It leads to situations where you never know what supplies you will need tomorrow.
With Th. Geyer as your comprehensive laboratory partner you can focus strictly on your research. As a family-owned company we are manufacturer-independent advisers and suppliers. We provide you with access to over 200 top brands, so you benefit in every way: you obtain everything you need for your laboratory from a single source – safely and reliably. This simplifies your processes and reduces your costs.
During presentation we will show you what tools can you exploit to go smoothly through collecting supplies for your next experiment.

Silver Sponsor Forum

O012 The usefulness of automated chromatography in protein purification

Stephan Pötsch

The interest in automated protein purification in academia and bioprocess is rapidly growing and the techniques have proven to provide gains in protein purity/productivity and savings in the cost of goods. The aim of this study is to demonstrate the usefulness of multistep chromatography and periodic counter-current chromatography (PCC) systems as suitable technological approaches, enabling continuous processing for both resin screening, scale-up studies, and unattended protein purification within the same systems. The advantage of dynamic control functionality in PCC runs with different sample concentration is shown.

Looking inside microbiology with Illumina next-generation sequencing O013

Piotr Kędziorski

ANALITYK GENETYKA illumina AUTHORIZED CHANNEL PARTNER

Next-generation sequencing (NGS) is opening new doors in microbial genomics, revealing fresh insight into how microbes impact humans and the environment. Through the power and high resolution of Illumina technology, we can now understand the genetic makeup of organisms that were previously impossible to study—helping to examine microbial biological functions, track genetic changes, rapidly respond to outbreaks, monitor food sources, and much more.

Introduction to Corning Life Science O014

Marcel Beckert

CORNING

Corning Life Sciences' laboratory products include general labware and equipment, as well as specialty surfaces, media, and reagents that are used for cell culture research, bioprocessing, genomics, drug discovery, microbiology, and chemistry. In your quest to deliver consistent, reproducible lab results, you need a partner you can count on. To supply you with a broad range of compatible lab consumables and equipment. To deliver them when and where you need them. To understand your science and your processes. To provide expert service and technical support. And to help support your lab's scientific goals.

Plenary Session – S-ystems

O015 From pure culture to multi-species communities: contributions of the biofilm concept to bacteriology

Jean-Marc Ghigo[1]

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In most environments bacterial colonization and survival is associated with the formation of dense bacterial communities called biofilms. While biofilms play many positive ecological roles, they are also difficult to eradicate when fouling medical and industrial surfaces Despite two decades of intense scrutiny, anti-biofilm approaches are still limited, as understanding the unique properties that differentiate biofilm from individual microorganisms still raises important scientific and technical challenges. I will discuss how exploring biofilm functions has contributed to help transitioning from classical pure culture studies to multispecies microbiology and to providing perspectives for biofilm control.

O016 The potential of long-read sequencing and bioinformatics for fungal and algal genome projects

Daniel Wibberg[1]

[1] FZ Jülich - ELIXIR Germany

Technological progress and the development of third-generation sequencing methods, e.g., Oxford Nanopore and PacBio SMRT sequencing, nowadays allow the rapid and cost-efficient high-throughput generation of complete genome sequences. Especially, Nanopore sequencing is a relatively inexpensive technology and is promising for genomes with high GC-content and highly repetitive sequences, since no PCR amplification is required for the preparation of Nanopore sequencing libraries and extreme long reads can be established. Single DNA molecules longer than a megabase can be sequenced using Nanopore, but the resulting sequence has a rather high error rate (usually in the 3–15% range). By means of sequence assembly and polishing of the consensus sequence with high depth Illumina reads, a similar error rate in comparison to short read data can be reached, but the assemblies include sequences spanning repeat regions and other regions that are not well defined with short read methods.
Thus, third-generation sequencing in combination with state-of-the-art bioinformatics displays a valuable tool for the investigation of phylogenomic and population genomic relationships for

up to now unknown fungal and algae families. In this talk, the genome projects of the fungal families *Hypoxylaceae* and *Ustilaginaceae* as well as the algal genus *Prototheca* will be presented as examples.

Virological surveillance during sanitary crises: sequencing tools and emergence detection

O017

Jean-Claude Manuguerra[1]

[1] Unité Environnement et Risques Infectieux, Institut Pasteur, Cellule d'Intervention Biologique d'Urgence (CIBU), Paris, France

A succession of epidemic crises has increasingly led to the rapid implementation of countermeasures. The level of information required from laboratories has increased enormously. In the 1980s, it was sufficient for human influenza national reference centers (NRC) to detect rapidly virus circulation and determine the type and subtype. Then, only a few NRCs also determined the current dominant variant after virus culture, which required several days. Later on, with the access to RT-PCR and sequencing, more laboratories were able to identify the season variant. This also took several days.
Suddenly in 2009, during the "Influenza A" pandemic and to detect the arrival in France of the new virus, it became necessary for the NRCs and CIBU to determine, in less than 10 hours, the type, subtype and whether it was the new A(H1N1)pdm2009 reassortant.
Following the large Ebola epidemic in West Africa (WA), the world health authorities needed to know asap if the 2016 DRC outbreak was linked to the 2013 WA epidemic. This would have been extremely bad news. In a few days, sequencing demonstrated that the outbreak was due to a local strain of Ebola with no direct link to that of Guinea.
Finally, the SARS-CoV-2 emergence and the succession of variants required their genomic monitoring. In November 2021, faced with the imminent arrival of Omicron in France, CIBU implemented emergency sequencing to obtain the complete viral sequence within 15 hours.
The trend is clear and concerns all countries of the world whatever their wealth or their infrastructures.

O018 From smear microscopy to whole genome sequencing: challenges of the laboratory diagnosis of tuberculosis in the 21st century

Miguel Viveiros[1]

[1] Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Portugal

Two hundred years after the birth of Louis Pasteur, the father of medical microbiology, and one hundred and forty years after the description by Robert Koch of the etiological agent of tuberculosis, *Mycobacterium tuberculosis* still is the leading cause of death by a single infectious agent worldwide. Early, rapid and accurate identification of *M. tuberculosis* and the determination of drug susceptibility is essential for the treatment and management of this disease. Tuberculosis diagnosis is mainly based on chest radiography, immune response tests, smear microscopy and bacteriological culture. Microscopy has low sensitivity and conventional culture for *M. tuberculosis* isolation, identification and drug susceptibility testing requires several weeks owing to the slow growth of *M. tuberculosis*. The delay in obtaining results promotes inappropriate antituberculosis therapy contributing to emergence of drug resistance and treatment failure. Novel diagnostic methods have been developed since 1985 for timely identification of *M. tuberculosis* and antibiotic susceptibility profile. Molecular methods offer enhanced sensitivity and specificity, early detection and the capacity to detect mixed infections and resistance related mutations, especially using whole-genome sequencing directly from samples. These technologies have improved turnaround time, cost effectiveness and are amenable for point-of care testing. However, phenotypic susceptibility testing is still needed for the accurate determination of drug susceptibility and to quantify the susceptibility levels towards individual antibiotics. Recent advances in the molecular diagnosis of tuberculosis and how the current phenotypic methods should be used in combination with the genotypic methods for rapid antituberculosis susceptibility testing will be presented and discussed.

Software tools and databases developed at the Institut Pasteur de la Guadeloupe to map global circulation of *Mycobacterium tuberculosis* complex genotypes

O019

David Couvin[1]*, Nalin Rastogi[1]

[1] WHO Supranational TB Reference Laboratory–TB and *Mycobacteria* Unit, Institut Pasteur de la Guadeloupe, France

For the last two decades, Institut Pasteur de la Guadeloupe has collaborated with various researchers to develop databases and software tools to facilitate our understanding of the world-wide circulation of *Mycobacterium tuberculosis* complex (MTBC) genotypes. These developments have been largely used by the scientific community to get an overview of tuberculosis (TB) molecular epidemiology based on PCR-based markers such as spoligotyping and MIRU-VNTRs. Several databases were developed over years. Recently released versions include SITVIT2 and SITVITBovis databases, the most recent version under development (SITVITEXTEND) contains information on 125,591 strains. These databases are based on the MySQL relational database management system and include genotyping data as well as available information such as species, year and place of isolation, origin, gender, and age of the patient (or identification of the animal host if relevant), drug-resistance, etc. Mappings and statistical analyses easily allow to interactively visualize and extract pertinent information from available epidemiological data. In parallel, we also focused on dedicated tools for phylogenetic analyses. For example, the recently developed SpolLineages tool allows the prediction of the MTBC families using classical and machine learning algorithms. Other genomic tools are in development to foster the extraction of peculiar genomic data, such as getSequenceInfo – a tool that will be helpful for the construction of whole genome sequencing (WGS)-based databases. Since MTBC WGS data is increasingly available, ongoing work also consists in the building of a bridge between WGS and genotyping data to study potential correlations and inferences on epidemiology, drug-resistance, and hopefully virulence factors.

Plenary Session – T-herapy

O020 Phage therapy: present and future

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Phage therapy (PT) offers realistic hopes in the fight against antimicrobial resistance. In the past years there has been a substantial increase in patients subjected to PT and the emergence of new PT centers in Europe and the USA. Furthermore, clinical trials are underway to confirm safety and clinical efficacy of PT in accord with the current requirements of Evidence-Based Medicine. The establishment, development and international recognition of our PT center has recently been discussed.

Our achievements on the international scene have contributed to the results of the recent ranking of scientific performance of all universities and research institutions in Poland made by the Ministry of Education and Science: our Institute has been awarded the highest rank (category A plus) in medical sciences – as the only Polish institution that has received such score.

Research of the past decade has shown that phages can interact with the immune system and the resulting alterations of its function could be exploited in the treatment of autoimmune diseases and other non-bacterial pathologies. These novel data can lead to phage application beyond their antibacterial action (phage repurposing). We just initiated studies funded by recently awarded non-commercial grant for a clinical trial of phage efficacy in the treatment of chronic bacterial sinusitis. Our studies should contribute to the establishment of the true applicability of PT in modern medicine.

O021 Development of novel anti-tuberculosis agents

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Despite significant progress in the development of new drugs against tuberculosis, many therapies and preventive measures for various reasons do not lead to the expected favorable health results. COVID-19, the global economy and high migration of people worldwide, HIV infection affect the incidence of tuberculosis and lead to increased resistance. These facts are a serious precondition for the selection of spontaneous mutations and the emergence of drug resistance to various anti-TB drugs. This opens the way to identify novel, structurally diverse compounds,

whose chemical, microbiological and pharmacological properties must be thoroughly elucidated prior to further development. We present the aroylhydrazone compounds regarding their: (i) acute and subacute toxicity in mice; (ii) redox-modulating capacity; (iii) pathomorphological observation in differentiated tissue specimens; (iv) intestinal permeability and (v) *in vitro* antimycobacterial activity. All compounds demonstrated significant minimum inhibitory concentrations (MIC) ranging from 0.07–0.32 μM, which were comparable to those of isoniazid. The screening identified 4-methyl-1,2,3-thiadiazole based hydrazone derivatives and sulfonyl hydrazones as new promising lead compounds against *Mycobacterium tuberculosis* H37Rv. Histological examination proved that the tissue findings do not show toxic changes. The *in vitro* antioxidant assays confirmed the results found *ex vivo*. The molecular docking performed in two crystallographic structures of enoyl-ACP reductase (InhA) displayed good docking scores and promising insights into possible interactions with the InhA receptor. These compounds display promising antitubercular drug-like properties and can be used for further investigation.
This work was supported by the Bulgarian National Science Fund (Grant KP-06-N41/3, 2020).

Fungi always have the last laugh: medical advances spoiled by fungal disease

O022

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We are living in a golden age of medical advances, but with every step forward comes a new peril, including opportunistic infections caused by fungi. For example, while organ and hematopoetic stem cell transplantation have changed the outlook for a myriad of diseases, fungal infections are a major scourge for these patients. Improvements in the understanding of pathogenesis enables therapeutic blockade of immunological cascades for various diseases, only to learn that these same pathways are important in protection against fungal disease. Notable examples include invasive mould disease and cryptococcosis with Bruton's tyrosine kinase inhibitors, and coccidioidomycosis and histoplasmosis with tumor necrosis factor alpha inhibition. Intensive care, surgical interventions, and antibacterials save countless lives each year but each also increase risks for invasive candidiasis. The importance of fungal diseases in spoiling medical and surgical successes demands both awareness among clinicians and greater societal investment in medical mycology.

Silver Sponsor Forum

O023 New diagnostic trends in molecular microbiology

Kasper Ciepluch

IMOGENA
Molecular diagnostics

O024 Microbiology in High-Content Screening images – modern assays with microorganisms

Marek Michałowski

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High-Content Screening (HCS) is a modern method for fluorescence and confocal microscopy cellular analysis. This technique is very useful for microbiological applications. HCS may be used for analysis of bacterial cells phenotyping and morphology, biofilm microenvironment and successful treatment research, living bacterial cells imaging, mammalian cell infection by bacterial cells or viruses monitoring etc. HCS is also useful in the area of biochemical and molecular microbiological studies.

HCS combines automated fluorescence, confocal and brightfield microscopy with automated quantitative image analysis, allows the acquisition of unbiased multi-parametric data at a single cell level. It is a fully automated technique. Each experiment is based on a detailed protocol (covering microscope set up, imaging, settings, image analysis, data extraction and mathematical presentation of the results) prepared by user.

Bacteriophage cocktails - a new sustainable solution for infectious agents O025

Elżbieta Fornal

Proteon Pharmaceuticals

Bacteriophages are as old as bacteria, as ubiquitous as bacteria and as natural as bacteria… Why? Because bacteriophages are the bacterial nemesis.

Bacteriophages were first successfully used to treat bacterial infections a decade before penicillin was discovered. They are perfectly natural bacterial antagonists, specific only towards their host strains and neutral for the remaining members of the microbiome.

Unfortunately, bacteriophages were severely underappreciated until the humanity has reached the dawn of the antibiotic era. In the face of an overwhelming threat from antimicrobial resistant strains, their unique mode of action, restricted to the pathogen and not the patient, cannot be overlooked.

Only now the exquisite features of bacteriophages, so opposite to antibiotics, are being explored. Unlike artificial chemicals, phages are nontoxic, completely biodegradable and the phage therapy triggers no side effects.

We should learn to draw from the benefits of bacteriophages' existence to increase the food safety, raise the bar on control of pathogens and most importantly improve our quality of life. This is the mission of Proteon Pharmaceuticals, which we have been introducing to the world for the past 15 years via our expanding/growing platform of high quality phage products for livestock.

The technology platform created by Proteon Pharmaceuticals allows to successfully develop phage-based products and implement new strategies of control of pathogenic bacteria affecting animal production industry. The platform allows to design highly effective products consisting of a mixture of carefully selected and genotypically characterized virulent phages that eliminate pathogenic bacteria, without causing side effects, while supporting better performance outcomes.

Plenary Session – E-ngineering & U-biquity

O026 Major evolutionary trends in clinical fungi

Sybren de Hoog[1]

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Black yeast-like fungi (order *Chaetothyriales*) are renowned for their ability to cause human infections. Extremotolerance is a major trend in the order, determined by ancestral rock colonization which required tolerance of toxic metabolites produced by lichens. This has shaped their current lifestyle, where endurance and efficient nutrient scavenging and toxin management come together. Since most species have to be traumatically inoculated to cause disease, their invasive potential is categorized as opportunism. In contrast to pathogens in, e.g. the dermatophytes (order *Onygenales*), lack of transmission from the infected host interferes with adaptation, and their infective ability is therefore unlikely to change over time. In contrast, dermatophytes, novel pathogenic species are continuously emerging. Possibly the black yeast disease chromoblastomycosis may be exceptional in showing a shift from opportunism to real pathogenicity, whereby transmission is key.

O027 Evolution of *Mycobacterium tuberculosis* complex

Roland Brosch[1]

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Tuberculosis caused by *Mycobacterium tuberculosis* remains one of the deadliest infectious diseases of humanity, even in the era of COVID-19. To better understand how *M. tuberculosis* has evolved into such a widespread and globally successful human pathogen, in our research unit for Integrated Mycobacterial Pathogenomics we focus on the evolution of different lineages of *M. tuberculosis* strains, as well as the very rare *Mycobacterium canettii* strains, which represent recombinogenic, early branching mycobacterial strains that are 98-99% identical in their genome sequences to *M. tuberculosis*, and members of a recently described new phylotype of closely related but non-tuberculous mycobacterial species. Genomic and phenotypic analyses and studies on inter-strain DNA transfer between the different strains have allowed us to reconstruct an evolutionary scenario in which the tubercle bacilli have evolved from low

virulent mycobacteria into a clonal complex of highly virulent pathogens of mammalian hosts. It is discussed how this evolution is linked to the gain of selected features as well as the loss of genetic material, both representing crucial factors that may influence the survival and persistence of tubercle bacilli in given hosts.

Mechanisms of solvent tolerance in *Pseudomonas* O028

Juan-Luis Ramos[1]*, Ana Garcia-Franco[1], Patricia Godoy[2], Estrella Duque[2]

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A number of microorganisms have the ability to thrive in the presence of a range of toxic solvents. Tolerance to these chemicals is a multifactorial process, meaning that bacterial cells use a set of physiological and gene expression changes to overcome the damage imparted by these chemicals. I will focus on issues related to tolerance to aromatic hydrocarbons and in *Pseudomonas*. *Pseudomonas putida* strains contain a circular chromosome of approximately 6 Mbp which encodes about 5300 genes. A combination of physiological and biochemical assays, a genome-wide collection of mutants and several omics approaches have provided useful information to help identify functions involved in solvent tolerance in *P. putida*. The solvent response involves fine-tuning of lipid fluidity to adjust membrane functions including impermeabilization, activation of a general stress-response system, increased energy generation and induction of specific efflux pumps that extrude solvents to the medium. These responses are modulated at the transcriptional level by local and global regulators as well as by a number of sRNAs whose levels fluctuate with the presence of solvents in the environment. Taken as a whole, these regulatory inputs orchestrate the complex network of metabolic responses observed after solvent addition.
Funded by: Ministerio de Ciencia e innovación RTI2018-094370-B-I00; European Commission: Grant agreement 862695 and 101070045; Junta de Andalucía: PAIDIP20_00049

O029 Cytosolic bacteria as platforms for innate immune signalling

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Invasive pathogens gain protection from canonical immune mechanisms of the host by occupying intracellular niches, but risk exposure to cellular defenses in those subcellular compartments. The cytosolic immune system is such a defense, and involves synchronized action of specialized signaling pathways to recognize the pathogen, resist invasion and inform other cells about ongoing infection. Interferon exposure enhances cell-autonomous immunity for efficient control of intracellular pathogens through the induction of numerous interferon-stimulated genes.
Remarkably, the interferon-induced GTPase family of guanylate-binding proteins (GBPs) sense cytosolic Gram-negative bacteria to transform the pathogen surface into a signaling platform. The GBP signaling platform initiates the death of infected cells and promotes inflammation by recruiting and activating the cytosolic lipopolysaccharide receptor, caspase-4. GBP-mediated activation of caspase-4 (i) eliminates infected cells before bacterial proliferation occurs, thus limiting the bacterial burden, and (ii) initiates processing of the pro-inflammatory cytokine IL-18, thus alerting immune cells to the presence of infection.
Moreover, GBPs coat bacteria in hierarchical manner reliant on GBP1, and inhibit actin-dependent motility and cell-to-cell spread of bacteria – key features of productive infection of *Shigella flexneri*, major human enteric pathogen. The GBP-mediated defenses are antagonized by IpaH9.8, a bacterial ubiquitin ligase secreted into the host cytosol. IpaH9.8 ubiquitylates GBPs causing the proteasome-dependent destruction of existing GBP coats, what prevents GBP signaling platform formation, caspase-4 activation, and liberates bacteria from GBP immobilization. Taken together, the function of GBPs is to transform the bacterial surface into an anti-bacterial signaling platform, whilst IpaH9.8 helps *Shigella* propagation by counteracting GBP dependent cell-autonomous immunity.

O030 The predominant clonal evolution (PCE) model of pathogenic microorganisms

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This model states that the impact of genetic recombination in pathogens' natural populations is not sufficient to erase (i) a statistically significant linkage disequilibrium (nonrandom association of genotypes occurring at different loci); (ii) a persistent phylogenetic signal at all evolutionary scales, from microevolution till geological times, in the whole ecogeographical range of the species considered. The clearest manifestations of this PCE pattern are the presence of deep phylogenies and multigene bifurcating trees.

We have applied this model to a set of representative bacteria, yeasts and parasitic protozoa species through the use of the most recent genomic data. All surveyed species, including those that were considered as highly recombining, such as *Neisseria meningitidis*, exhibit similar PCE patterns above and under the species level, from macro- to micro-evolutionary scales ("Russian doll pattern"), which suggests gradual rather than, saltatory, evolution. PCE does not state that sexual reproduction is absent, but rather, that it is not frequent enough to break up the pattern of predominant clonality. It does not state either that sexual reproduction has no epidemiological or evolutionary relevance. To our knowledge, this is the first time that such a strong common evolutionary feature among very diverse pathogens has been evidenced. The implications of this model for basic biology and applied research will be exposed.

Gold Sponsor Forum

The role of education in building awareness of infectious diseases among doctors and patients

O031

Pawel Pacholczyk

sanofi

We have a lot of possibilities to learn new things, but a gap in our education is still difficult to imagine.
One of the roles of a pharmaceutical company should be raising awarness of diseases, demonstrating newest data and being in contact with KOLs to collect insight from them.
In Sanofi Pasteur our mission is even wider (in comparison to typical Rx products) – we should also teach patients (not only HCPs).
What kind of initiatives do we (medical department) have? We try to to be part of the biggest national conferences, not only to present but also to listen what kind of problems are the most important.
To be more practical – very frequent educational activities are webinars organised for smaller groups of doctors.
This kind of digital tool started to be more popular during and after COVID-19 pandemic, I am sure that it works really very well.
As I mentioned at the beginning, we have had also many initiatives for patients – they must trust science and be sure that protection is the key to success of minimalizing infectious diseases.
Our education should be holistic and have the same consistent message – only in this way we can create positive attitude from patients and all the staff.
The example of our wide perspective is #mamyodpornosc campaign. We started talking about the need for vaccination of pregnant women with gynecologists, later we approached GPs. Next year we will talk to midwives and women.

Plenary Session – R-esistance

O032 How integrons helped Gram-negative bacteria to overcome the antibiotic threat

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Integrons are mainly known as the genetic agents responsible for the capture and spread of antibiotic resistance determinants among Gram-negative pathogens, spread horizontally by plasmids. They are also found in the chromosomes of hundreds of environmental bacterial species, where cassettes convey much broader adaptive functions. These chromosomal integrons are thought to be the sources of both the antibiotics resistance cassettes and the integron platforms that convey these cassettes among bacterial pathogens.
I will present the refinements of this genetic device that explain why integrons have been so successful in allowing Gram-negative pathogens to resist antibiotic treatment.

O033 On the emergence and drug resistance of *Mycobacterium tuberculosis*

Philip Supply[1]

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Rapid, comprehensive diagnosis and surveillance are key to control and manage drug resistance, which is especially crucial for tuberculosis (TB). Multi-/rifampicin drug resistant (MDR/RR) TB is the single largest contributor to human mortality due to antimicrobial resistance. Only one in three of the estimated 500,000 people who develop MDR/RR-TB each year are diagnosed and started treatment. Phenotypic resistance profiling is slow, and conventional molecular tests for detecting *Mycobacterium tuberculosis* (MTB) drug resistance interrogate a limited set of common mutations. This presentation will provide an overview of next-generation sequencing (NGS) approaches developed to fill this diagnostic gap. An extensive WHO-endorsed catalogue of drug resistance mutations was constructed by whole genome sequencing (WGS) of 38,000 isolates from global sources. In order to by-pass the need for mycobacterial culturing before WGS analysis, we developed with GenoScreen a targeted deep NGS assay, named Deeplex Myc-TB, directly applicable to primary specimens. Coupled with a web app integrating the WHO mutation catalogue, this assay allows simultaneous prediction of (hetero)resistance to 13 anti-TB drug classes, including those now defining extensively drug resistant TB, as well as MTB/NTM identification and MTB genotyping for molecular surveillance. This comprehensive molecular

diagnostics enabled us e.g. to detect a longitudinal MDR-TB outbreak and nationally prevalent cases of zoonotic TB, both undetected by WHO-endorsed tests. By combining this hi-plex amplicon sequencing approach with comparative genomics, we also discovered new ancestral strain lineages in East Africa, providing unique proximal insights into the origins and emergence of the pathogen.

Spread of carbapenemase-producing Enterobacterales in Poland before and during the COVID-19 pandemic

O034

Marek Gniadkowski[1]*, Waleria Hryniewicz[1], Elżbieta Literacka[1], Dorota Żabicka[1]

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Over the last two decades antimicrobial resistance (AMR) in pathogenic bacteria has been recognized as a critical threat for public health, and the COVID-19 pandemic has raised a great deal of concern about its possible long-term negative impact on AMR. Recently this has been confirmed by a CDC 2022 Special Report with the US 2019–2020 data on AMR infections. According to the National Reference Centre for Susceptibility Testing (NRCST), Poland apparently belongs to the countries where the AMR epidemiological situation has remarkably worsened during COVID-19. It has been documented by the 2021 surveillance data for carbapenemase-producing Enterobacterales (CPE), pathogens of the highest clinical and epidemiological relevance. In 2021 the NRCST recorded 4,172 non-duplicated CPE isolates, compared to 2,064 in 2019, which was a ~100% increase. This was largely due to the NDM-type CPE, the number of which doubled from 2019 to 2021 (n = 1,527 and 3,036, respectively), and accounted for ~73% of all CPE samples. In the case of KPC CPE, the 741 cases recorded in 2021, when compared to 247 in 2019, indicated a dramatic ~200% increase. Finally, the OXA-48-type CPE reached the number of 285 cases in 2021 versus 170 in 2019, an increase of ~70%. These observations correlate well with the ECDC EARS-Net data on carbapenem resistance in invasive *Klebsiella pneumoniae* isolates from 52 Polish hospitals. The rate has radically grown from 7.7% in 2019 to 19.5% in 2021, confirming clearly the strong negative impact of the COVID-19 pandemic on AMR in Polish medical centers.

O035 The interplay between climate change, drug resistance and new infectious diseases

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The world is getting warmer and microbes are adapting to higher temperatures. We know that the geographic range for microbes and disease vectors will change but relatively little attention has been given to the effect of temperature on virulence. Virulence is a microbial property that is expressed only in a susceptible host. Mammals are remarkably resistant to disease with many microbes with pathogenic potential because of a combination of high basal temperatures (endothermy) and an advanced immune system that includes adaptive immunity. Of the more than 6 million fungal species only about 150-300 are pathogenic for humans, and of these, only 10-15 are relatively common pathogens. Hence, humans have tremendous innate resistance to fungal diseases, and this contrasts sharply with the fact that fungi are major pathogens for plants, ectothermic vertebrates and insects. In this regard, vertebrate endothermy and homeothermy create a restricted environment for most fungal species. However, as microbes adapt to higher ambient temperatures, they could acquire thermal tolerances that allow it defeat mammalian endothermy. Such an example could be *Candida auris*, which emerged simultaneously in three continents, already drug resistant. Thinking backwards in time, I will present the hypothesis that fungal diseases contributed to both the extinctions at the end of the cretaceous and to the great mammalian radiation that followed in the tertiary era. Thinking forward in time, the seminar will consider possible consequences of climate change, which include the emergence of new infectious diseases as microbial species adapt to a warmer world.

Silver Sponsor Forum

O036 Challenging AMR with antibiotic stewardship

Ernesto Battinelli

ThermoFisher SCIENTIFIC

Antibiotic stewardship is the effort to measure and improve how antibiotics are prescribed by clinicians and used by patients. Improving antibiotic prescribing and use is critical to effectively treat infections, protect patients from harms caused by unnecessary antibiotic use, and combat antibiotic resistance. This lecture will show ways how to effectively combat AMR using today's available measures.

Plenary Session – Miscellanea

Genetic variability, genotyping and genomics of *Mycobacterium leprae* O037

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Amongst *Mycobacterium* species, *M. leprae* and *M. lepromatosis* cause leprosy and are considered genetically stable. In the past millennium, hardly any data existed on morphologic or genetic differences on a strain level and even today, these species are considered particularly stable. Combine this with the difficulty to replicate *M. leprae in vitro* and it is not surprising that the development of genotyping procedures is relatively recent. There has been considerable progress after obtaining the first genome sequence, leading to better understanding of the biology of *M. leprae*. Sequencing a second genome and larger scale sampling allowed comparative genetic analysis leading to better understanding of human migration patterns and spreading of the parasite on a global level. Detection of SNPs and VNTRs is the basis of (standardized) genotyping allowing a better elucidation of lineage definition, bacterial population structure, strain transmission and reinfection. Molecular basis for drug resistance in leprosy is partly uncovered and is now part of surveillance in several endemic countries. A recently developed t-NGS-based typing technique (Deeplex® Myc-Lep) allows detection of DR and is being used in molecular epi studies. Leprosy is not a strictly human disease and some other mammalians have been demonstrated to develop the disease. In the US, the armadillo is participating in leprosy transmission and the extent to which a one health approach is needed in endemic countries needs to be investigated. Laboratory infection of ticks and kissing bugs with *M. leprae* has been demonstrated but their participation in transmission of disease has not really been elucidated.

O038 Modern Koch's postulates applied to bacterial pathogenesis of Alzheimer's disease

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In the 19th century Robert Koch established rigorous guidelines to evaluate causation in infectious disease. These postulates have been updated to reflect modern scientific methods including DNA sequencing and immunohistochemical techniques. First, a DNA/protein belonging to a putative pathogen should be present in most cases of an infectious disease; second, lower level, or none pathogen-associated DNA/antigens should occur in hosts or tissues without disease; third, with effective treatment, the level of pathogen-associated factors should decrease. Applying these postulates to *Porphyromonas gingivalis* revealed that this bacterium may contribute to the pathogenesis of Alzheimer's disease (AD) through the activity of gingipains, its main virulence factors. First, both *P. gingivalis* derived DNA and gingipain antigens were identified in the brain of AD patients. Notably, a level of gingipains in the brain of AD patients correlated with tau and ubiquitin pathology. Second, although gingipains were found in the brain of age-matched controls, they occurred at a significantly lower level. Third, oral infection of mice with wild-type *P. gingivalis*, but not gingipains-null strains, resulted in brain colonization and increased deposition of Aβ1-42 plaques. Fourth, gingipains were neurotoxic *in vivo* and *in vitro*. Fifth, targeting gingipains by small-molecule inhibitors in a murine model diminished the bacterial load of an established *P. gingivalis* brain infection, blocked Aβ1-42 production, reduced neuroinflammation and rescued neurons in the hippocampus. Together these results revealed the potentially causative relation between chronic periodontitis and AD and suggested that gingipain inhibitors could be valuable for treating *P. gingivalis* brain colonization and neurodegeneration in Alzheimer's disease.

Silver Sponsor Forum

Microbiological and molecular diagnosis of *Mycobacterium tuberculosis* infections

O039

Tomasz Szczęsny

BIOMAXIMA

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. According to WHO, the number of tuberculosis cases fell by nearly 20% from 7.1 million in 2019 to 5.8 million in 2020. In addition, the number of patients treated for drug-resistant tuberculosis decreased by 15%, from 177 000 in 2019 to 150 000 in 2020. In Poland, the incidence of tuberculosis is relatively low (13.9 cases per 100 000 in 2019). In 2019, more than 5300 cases of tuberculosis were registered in Poland, 166 cases less than in 2018. The incidence of tuberculosis in 2019 was 13.9; decreased by 29.4% compared to 2010, when it amounted to 19.7. The incidence of tuberculosis was 8.8 in 2020, which is a decrease of 36.7% compared to 2019. Currently, it seems particularly important to track the spread of mycobacteria in our eastern neighbors. In Ukraine in 2019 there were more than 28 000 cases of tuberculosis, the incidence was almost 65. In the same year, more than 2600 cases were reported in Belarus, and the incidence was over 27. How can this affect the current and future epidemiological situation in Poland? Certainly, early detection and diagnosis of patients prevent the transmission of drug-resistant tuberculosis into the environment. At what level of healthcare organization do we diagnose tuberculosis in Poland? What methods do laboratories have for the rapid and accurate diagnosis of *Mycobacterium tuberculosis* infections?

Corpus Christi procession in Castle Square, Warsaw, 1890s.

Photo by Konrad Brandel, The Museum of Warsaw.

Poster Session (P)

| Topic categories |

I. P-athogens

Microbial Pathogens of Man: Taxonomy – Virulence – Infectious Diseases – Epidemiology – Diagnostics – Host-Pathogen Interactions

P001 The impact of the COVID-19 on tuberculosis burden in Kosovo

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Since being declared a global pandemic, Coronavirus disease 2019 (COVID-19) prompted the interest of healthcare providers and researchers. This study aims to evaluate the impact of the COVID-19 pandemic on tuberculosis management and incidence in Kosovo.

The retrospective survey was conducted at the Department of Microbiology within the National Institute of Public Health of Kosova. The sputum smear for acid-fast bacilli, Lowenstein-Jensen medium for culturing *Mycobacterium tuberculosis* and GeneXpert MTB/RIF (Cepheid Sunnyvale, CA, United States) during 2020 were used for tuberculosis (TB) diagnosis during the survey period.

The first case of COVID-19 in Kosovo was detected on March 13, 2020, followed by a rapid increase in the number of cases thereafter. The total number of cases confirmed by the middle of June 2022 was 228,689, with 3,140 deaths among the total population of 1,780,000.

There was a gradual decline of the TB notification rate per years from 2015 – 770 cases /100,000 population; 2016 – 675 cases/100,000; 2017 – 656 cases/100,000; 2018 – 654 cases/100,000; 2019 – 545 cases/100,000 until 2020 – 441 cases/100,000.

We found a marked decline in the incidence rate of TB in Kosovo during 2020, the chi-square test showed that the value of 441 cases /100,000 inhabitants was significantly lower than expected when compared to the trend from previous years (p=0.01).

We find highly significant influences between COVID-19 pandemic and the incidence of TB in Kosovo. There have been substantial disruptions to TB health services and an increase in vulnerability to TB. Lockdown, social distancing, isolation strategies and public health guidelines to prevent viral transmission impacted the delivery of all aspects of TB care.

Detection of resistance to second-line drugs in *Mycobacterium tuberculosis* using *in silico* approaches

P002

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In 2021, *ca.* half a million people developed tuberculosis (TB) eligible for treatment with second-line drugs (SLDs). Yet, relatively few studies evaluated the use of whole-genome sequencing (WGS) in prediction of TB susceptibility to SLDs.

The aim of the study was to compare the capacity of two *in silico* WGS-based approaches for the detection of resistance to SLDs in *Mycobacterium tuberculosis*.

The study included 118 multidrug-resistant (MDR) and 60 drug-susceptible (DS) isolates, recovered from as many (178) Polish and Lithuanian patients between 2018 and 2021. Conventional drug susceptibility testing was performed using BACTEC MGIT 960. WGS was done with Illumina NovaSeq 6000 sequencer. Molecular determination of resistance to amikacin (AMK), capreomycin (CAP), kanamycin (KAN), moxifloxacin (MOX), and ofloxacin (OFX) was done with Mykrobe and TBProfiler. The latter application was also used to assess resistance to bedaquiline (BDQ), delamanid (DLM), ethionamide (ETH), and linezolid (LZD).

Both tools produced congruent results for all tested drugs, expect for OFX and MOX. For those two fluoroquinolones the concordance of the results between phenotypic and genotypic assays was higher for Mykrobe (93.3% for OFX and 94.9% for MOX) than for TBProfiler (77.5% for OFX and 89.3% for MOX).

Overall, the sensitivities of the *in silico* approaches varied across drugs, and were the highest (100%) for MOX (assessed with Mykrobe), and the lowest (50%) for OFX (with TBProfiler), MOX (with TBProfiler), LZD, and DLM.

Given the relatively low sensitivities of Mykrobe and TBProfiler for the detection of resistance to SLDs in *M. tuberculosis*, the performance of standard phenotypic tests is advised.

P003 Entry and transport of mouse coronavirus (MHV-JHM) in neurons: the role of the cytoskeleton

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Nowadays, the population is still struggling with a post-COVID19 syndrome known as long COVID, including a broad spectrum of neurological problems. There is an urgent need for better understanding and exploring mechanisms of coronavirus neurotropism. For this purpose, the neurotropic strain of mouse hepatitis virus (MHV-JHM) originating from the *Betacoronavirus* genus, same as SARS-CoV-2, was used. The cellular receptor and role of cytoskeleton during virus replication in neurons *in vitro* were determined to understand the mechanisms of MHV-JHM neuroinfection. Viral antigen, receptors, actin cytoskeleton, and cell nuclei were detected by indirect immunofluorescence and fluorescent staining. The impacts of modulating compounds (actin, microtubule, and serine proteases – furin & TMPRSS2 inhibitors) on viral replication were detected by RT Real-Time PCR. For further analysis, Olympus FV10i confocal microscope and ImageJ software were used.

We discovered that the MHV-JHM strain utilized the actin and microtubule cytoskeleton of neurons during infection. Observed were: (i) condensation of actin filaments in the cortical layer of the cytoplasm (2 and 24 hpi); (ii) presence of viral antigens along stress fibers; (iii) formation of long actin structures used by MHV-JHM for intercellular transport (24, 48, and 168 hpi), (iv) syncytia (since 48 hpi) and plague (168 hpi) formation. During RT-PCR analysis, several used compounds showed inhibiting antiviral activities. In neurons, we observed for the first time that MHV-JHM utilizes both cellular receptors – CEACAM1 and PSG16; however, colocalization analysis performed by the ImageJ JACoP BIOP tool revealed a dominant role of PSG16 during neuronal infection.

Genetic diversity of multidrug-resistant and drug-susceptible *Mycobacterium tuberculosis* isolates in Poland

P004

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Poland ranks 6th in terms of the highest TB incidence among the European Economic Area countries. The objective of this study was to explore the genetic diversity of multidrug-resistant (MDR) and drug-susceptible (DS) *Mycobacterium tuberculosis* isolates from Poland with a combination of spoligotyping and mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) analysis.

The study included 89 (39 MDR and 50 DS) *M. tuberculosis* isolates collected from as many patients between 2018 and 2021 in Poland. Spoligotyping was carried out with a commercially available kit. MIRU-VNTR analysis was done at 24 standard and 4 hypervariable (HVL) loci. Phylogenetic clades of *M. tuberculosis* were assigned according to SITVIT2.

In total, 39 spoligotypes were identified, split into 11 clusters (n = 61, 68.5%, 2–19 isolates per cluster) and 28 (31.5%) unique patterns. Most isolates belonged to the Beijing family (n = 25; 28.1%), followed by T (n = 24; 27.0%) and Haarlem (n = 14; 15.7%) families. Among MDR *M. tuberculosis* isolates, nearly 60% (23/39) were of Beijing genotype.

A combined spoligotyping and MIRU-VNTR analysis resolved 5 clusters (n=18, 20.2%, 2–6 isolates per cluster) and 71 (79.8%) unique patterns. Upon MIRU-VNTR 24 loci analysis coupled with HVL, no clustered isolates were observed.

In conclusion, almost 70% of Polish *M. tuberculosis* isolates belonged to 3 molecular families, i.e. Beijing, T, and Haarlem. Whereas the Beijing family was the most prevalent (60%) among MDR-TB cases, it accounted for only 4% of DS isolates. The incidence of TB in Poland seems to be attributable to reactivation of latent infection rather than recent transmission.

P005 Detection of *Streptococcus agalactiae* among pregnant women with an application of CDC protocol

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*Streptococcus agalactia*e, an important human opportunistic pathogen, belongs to beta-hemolytic *Streptococcus* spp. representatives. This pathogen accounts for a significant part of early infections in pregnant women and neonates, including serious life-threatening infections. This research investigated the usefulness of Centers for Disease Control and Prevention (CDC) protocol for *S. agalactiae* DNA detection in 250 samples of recto-vaginal swabs collected from pregnant women (at 35–37 weeks of gestation) and pre-cultured overnight in Todd-Hewitt broth. With an application of the CDC protocol-based real-time PCR, the *cfb* gene was detected in 68 (27.2%) samples, compared to 41 (16.4%) for the standard reference culture-based methodology. The applied molecular method presented high sensitivity (100.0%) and specificity (87.1%). Therefore, it allowed for more precise detection of *S. agalactiae*, compared to the reference diagnostic method. The increased sensitivity of GBS detection leads to more reasonable antimicrobial prophylaxis therapy of GBS infections in pregnant women and may result in a reduced number of infections in newborns. In addition, the use of this molecular method allows for a significant reduction in the turnaround time for GBS detection, while an interpretation of the results is relatively simple. Therefore, it enables a faster intervention in case of a necessity of an antibiotic therapy introduction at least in a group of pregnant women whose GBS status is unknown at the time of delivery.

P006 Protein and lipid profiles of extracellular vesicles of *Cutibacterium acnes*

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Cutibacterium acnes, previously *Propionibacterium acnes*, represents human skin microbiota. These bacteria are also responsible for opportunistic infections. In *C. acnes* three major phylotypes are found, and are associated with different clinical conditions, e.g. type IA1 dominated in isolates cultured from acne-affected regions, type IB in isolates from soft tissue infections.
Extracellular vesicles are small, closed bilayer nanostructures which are purposely secreted by bacteria to aid in communication and contribute to numerous bacterial functions. EVs originate from bacterial cells in a different manner depending on bacterial cell wall properties. EV are composed of proteins, lipids, and may contain nucleic acids.
The aim of the studies was elaboration of proteins and lipids content of EVs secreted by *C. acnes* phylotypes: IA, IB, II and III, and their comparative analysis.

The *C. acnes* spp. were cultivated in tryptic soy broth-thioglycolate medium in anaerobic conditions using Gas Pack system. EV were isolated from the medium using density gradient ultracentrifugation. The SDS-PAGE protein profile and the lipid profile were studied using the TLC and MALDI-TOF MS. Purified EVs of *C. acnes* spp. were in the same range, the diameter was about 100 nm, the biggest EVs were from phylotype III. The sizes of EV proteins obtained from various *C. acnes* strains were different. Lipid profile revealed that the majority of lipids were common and a few distinct lipids were also found.
C. acnes phylotypes I, II, III, besides belonging to the same species, release distinct EVs. Further studies on the biological significance of these compounds are needed.

The first study on skin microbiota in prurigo nodularis P007

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Prurigo nodularis (PN) is a chronic skin disease, debilitating condition characterized by the presence of multiple nodular lesions accompanied by intense pruritus. The exact etiology of the disease is not fully elucidated. The data on the role of infectious factors in the development of PN is scarce. So far, the bacteria in lesional skin have been identified using classical isolation techniques. The availability of next generation sequencing (NGS)-based techniques using 16S rRNA analysis has revolutionized the approach to the role of microorganisms in numerous dermatoses. Dysbiosis, rather than one specific pathogen, is believed to be responsible for the development of many skin diseases.
The aim of this study was to evaluate the microbiota in PN lesions by targeting the V3-V4 region of 16S rRNA. Skin microbiota samples for the analysis were collected at the Department of Dermatology, University of Rzeszów. To the best of our knowledge, this is the first study on the skin microbiota in PN.
Our preliminary results point to significant differences in the composition of the skin microbiota between patients with PN and healthy volunteers (HV). The skin microbiota of PN patients showed a reduction in microbial diversity. The most significant result is an increase in the abundance of the genus *Staphylococcus* in the PN microbiota (84%), associated with a significantly lower abundance of other bacteria. Understanding these complex interactions between skin microbiota in PN may be an important step in developing target treatments with prebiotics and probiotics.
Research funded by the Podkarpackie Innovation Centre grant (N2_049).

P008 Hypersensitivity to alkaline pH contributes to a unique feature of *Cryptococcus neoformans/gattii* species complex to form Titan cells

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Members of the *C. neoformans/gattii* species complex are unique among basidiomycetous yeasts in their ability to cause lethal meningitis in immunocompromised and occasionally also immunocompetent individuals. Among the virulence factors contributing to cryptococcosis is poorly characterized transformation into Titan cells, a feature that prevents phagocytosis by host macrophages. Based on protocols to obtain Titans *in vitro*, it has been proposed that this morphological transition is unique to *C. neoformans/gattii* species complex. To explain what makes those species capable of undergoing such an unusual morphological change, we defined minimal conditions necessary to obtain Titan cells *in vitro*. We found that exposing stationary phase cells to PBS with pH 7.3, and supplemented with 0.05% glucose, 0.025% $(NH_4)_2SO_4$, 0.004% K_2HPO_4, 0.0035% $MgSO_4$, in the presence of 5% CO_2 at 37°C triggers Titan cell formation to the same degree as the known protocol that utilized 10% fetal bovine serum (FBS) as the sole nutrient source. Interestingly, we found that optimal pH of the media supporting formation of Titans was around 7.3. Relatively acidic pH prevented this morphological transition and promoted proliferation, whereas alkaline pH caused excessive growth inhibition and prevented Titan cell development. Strikingly, we found that members of the *Cryptococcus neoformans/gattii* species complex are more sensitive to alkaline pH as compared to other basidiomycetous yeasts. Our findings suggest that, opposite to other members of *Cryptococcus* anamorphic genus, the unique response to slightly alkaline pH accounts for the ability to form Titans in *Cryptococcus neoformans/gattii* species complex, which contributes to pathogenesis.

Assessment of the incidence of streptococci and *Haemophilus* spp. in the course of exacerbations of chronic obstructive pulmonary disease – preliminary studies

P009

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Chronic obstructive pulmonary disease (COPD) is a chronic disease and has a progressive course, especially when there is constant exposure to lung damaging factors. The presence of some species of bacteria, including *Streptococcus pneumoniae* or *Haemophilus influenzae* contribute to the occurrence of COPD exacerbations. However, none of the mechanisms underlying the development and exacerbation of the disease are yet known.

The aim of the research is to analyze the presence of streptococci and *Haemophilus* spp. in the oral cavity, respiratory tract and large intestine in patients suffering from COPD, especially during exacerbations of the disease.

The research material consisted of oral swabs, sputum and faces collected from patients with COPD exacerbations. Cultures were grown on CNA for streptococci and chocolate batracin agar for *Haemophilus* spp. Cell abundance per 1ml of sputum and 1g of faces was determined. Species identification was performed using APIStrep and APINH biochemical tests.

23 patients participated in this stage of the study. Streptococci were isolated from 6 and *Haemophilus* spp. also from 6 patients. Both streptococci and *Haemophilus* spp. were isolated from 4 patients. Detailed results regarding abundance and species identification will be presented during the conference.

This study is part of a large project to link selected microorganisms to COPD markers to identify the mechanisms responsible for the occurrence of exacerbations in the disease course. Understanding the impact of microorganisms on COPD progression and the risk of exacerbations will lead to the development of targeted therapies to prevent COPD progression and exacerbation.

Approval of the Bioethics Committee no. RNN/181/21/KE.

P010 Influence of the third dose of COVID-19 vaccination on the dynamics of anti-SARS-CoV-2 IgG antibodies

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Almost three years ago, the COVID-19 pandemic broke out, causing serious new health, diagnostic, therapeutic and socio-economic problems. Its symptoms are weakening slowly and people are getting used to the presence of SARS-CoV-2. However, anxiety about the post-infection effects and further preventive and therapeutic solutions is not ceasing. The upcoming autumn-winter season causes concern about the growing morbidity and questions whether to introduce further anti-epidemic restrictions and booster vaccinations. In our project, we provide new evidence on the dynamics of the anti-SARS-CoV-2 spike protein IgG levels before and after the third dose of vaccination against COVID-19 among 93 participants. The IgG antibody levels were tested at four time points according to age, gender, COVID-19 history, medical profession, and baseline antibody levels. We have shown that after the third dose of vaccination, the IgG levels were significantly higher and more stable than after the second dose. It is advisable to apply booster vaccinations in the case of borderline IgG because the immune system will be more effective then. We have also shown that antibody levels positively correlate with female and healthcare workers, also, surprisingly, with the elderly (over 60 years of age) and people with negative COVID-19 history. The preliminary results (23 participants) showed that antibody levels dropped 1.5-fold after 4 months from the last measurement but remained at a very high level. The research results obtained indicate the validity of using booster vaccinations against COVID-19 and deepen the knowledge about the interaction of the host – SARS-CoV-2 virus.

P011 The role of Hfq protein in post-transcriptional regulation of iron uptake in *Yersinia enterocolitica* 2/O:9

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In *Yersinia enterocolitica* and other Gram-negative bacteria, the concentration of iron in the environment is critical for controlling bacterial metabolism, and iron-acquisition systems are considered essential pathogenicity determinants. Hfq is a chaperone protein that promotes the binding of sRNA to its target mRNA resulting in, among other things, translation blockade or transcript downregulation.

This study aimed to investigate the effect of Hfq on the post-transcriptional expression of *Yersinia enterocolitica* biotype 2/O:9 genes related to iron assimilation, i.e., *fur*, which encodes a repressor of iron transport systems, *fecA* and *fepA*, encoding receptors for Fe-siderophores.
Translational plasmid fusions of the selected genes with *gfp* encoding green fluorescence protein (GFP) were used. Fluorescence of the fusion proteins: Fur'-'GFP, FecA'-'GFP and FepA'-'GFP was analyzed in strains differing in the activity of Hfq protein, i.e., in *hfq*-deletion mutant and wild-type strain. Cultures were cultivated in medium supplemented with additional iron source or iron chelator, to the exponential and stationary phase.
The study showed that Hfq inhibits the expression of *fur*, *fepA*, and *fecA* of *Y. enterocolitica*, most likely at the post-transcriptional level. Similar results were obtained in all provided conditions at both growth phases.
We demonstrated that the Hfq protein by modulating Fur, FecA, and FepA levels, regulates iron assimilation in *Y. enterocolitica* and thus the proper functioning of the bacterial cell.

The pathogenic equipment of coagulase negative staphylococci, its regulation and cross-talk

P012

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The ability of staphylococci to occupy numerous host niches, behave either as a commensal or a pathogen, has been attributed to the regulation of colonization and virulence factors. Although for many years only *Staphylococcus aureus* was considered pathogenic among the staphylococci group, also coagulase negative staphylococci (CoNS) may contain similar genes or the ability to acquire genes from related species by horizontal gene transfer and thus, may also be an etiological factors of infections. Staphylococcal virulence factors are regulated by transcriptional regulators of two-component systems and quorum sensing systems, including accessory gene regulator (*agr*). The aim of our studies was to check if clinical CoNS strains, which were confirmed as etiological factors of chosen human infections, may contain virulence factors such as hemolysins and genes involved in biofilm formation as well as elements responsible for regulation of genes that encode virulence factors typical for *S. aureus*. The presence and activity of virulence factors that are homologous to *S. aureus* were tested phenotypically and genotypically. Moreover, we checked whether the regulation factors produced by one CoNS isolate can affect the virulence activity of other strains. Our studies confirmed the presence of a virulence factor and regulatory genes attributed to *S. aureus* in CoNS isolates as well as indicated that one strain with active *agr* gene is able to affect biofilm formation and δ-toxin activity of strains with inactive *agr* genes.

P013 Mixed anaerobic skin infection in an injection drug user. A case report

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Bacterial skin and soft tissue infections among drugs abusers predispose to serious health consequences. Little attention is being paid to these problems.
We described a case of chronic wound infection related to the injection of the drug caused by anaerobes considered as bacteria with low pathogenic potential, inhabiting gastrointestinal tract of healthy people.
A 42-old male was admitted to the ED because of an infiltration on the inner side of the thigh below the scrotum at the site of a needle insertion for drug administration. The USG showed a solid-fluid hematoma in the muscular layer (length 13 cm).
On the penile base, a heterogeneous, indistinctly demarcated area of the litho-fluid lesion with the features of an abscess with segmental flow within the lesion and with gas bubbles was also detected. CRP was 144.12 mg/l. *Slackia exigua*, *Atopobium parvulum* and *Parvimonas micra* were isolated from the skin lesions.
Case reports published over the past few years revealed that *S. exigua* is associated with sepsis and periprosthetic joint infection. *A. parvulum* has been isolated from the oral cavity of healthy persons, but also those with odontogenic infections. *P. micra* is a part of the microbiota of the gastrointestinal tract and can also cause bloodstream infections, spinal infections, and sepsis.
Currently new methods of bacterial identification are changing the face of medical microbiology. Species not previously associated with humans or considered non-pathogenic are increasingly identified. We should not ignore it in the routine diagnosis of infections, especially in non-immunocompetent patients.

P014 Prolonged treatment of *Salmonella enterica* strains with human serum affects their basic phenotype

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Salmonella enterica are good model organisms to conduct research on bacterial biology. These bacteria can multiply in external environments but also in living hosts, which proves their wide adaptability. *S. enterica* have been serially passaged in human serum from platelet-poor plasma (SPPP) to induce a series of phenotypic changes as a response to unfavorable conditions. For this purpose, resistance to antibiotics, biofilm formation, motility, and membrane protein (MP) profiles have been studied. The obtained results confirm *Salmonellae* adaptation to SPPP through

many acquired characteristics. *S. enterica* serovar Hammonia developed resistance to colistin, *S.* Typhimurium ATCC 14028 and *S.* Senftenberg became moderate biofilm producers from strong biofilm producers. Passages in SPPP also resulted in swimming motility, where *S.* Typhimurium ATCC 14028 were significantly less motile in contrast to *S.* Erlangen which showed greater motility. Moreover, we have observed the opposite changes within MP patterns (SDS-PAGE), especially in *S.* Hammonia and *S.* Typhimurium ATCC 14028. Since *Salmonella* Hammonia adaptants produced more MPs, in *Salmonella* Typhimurium ATCC 14028 fewer protein bands were detected. The above observations suggest that adaptation of *S. enterica* to stressful conditions manifests on many levels. On top of it all, colonial morphotypes of the adaptants were similar to those produced by starting cultures. This is an example in which stable morphotypes distinguished by altered virulence can be confusing during laboratory work with life-threating strains. This work was partly supported by program „Excellence initiative – research university" for years 2020–2026 for University of Wroclaw, Poland.

The effectiveness of monomeric quaternary ammonium salts differing in chain length against pathogenic bacteria

P015

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Quaternary ammonium salts (QAS) are especially important as active ingredients in disinfectants. Studies have shown their effectiveness against Gram-positive bacteria, Gram-negative bacteria, fungi and enveloped viruses. QAS show bactericidal activity against both planktonic forms and biofilms. In addition, with the electrostatic interactions between the compound and the abiotic surface, they have the ability to coat it. Thus, QAS could find application as compounds that prevent the adhesion of microorganisms to an abiotic surface. Such use would prevent the formation of harmful biofilms on the surfaces of catheters, surgical instruments, endoprostheses or artificial valves. A group of three monomeric QAS with methylcarbonate counterions differing in aliphatic chain length (C12, C14, C16) against Gram-positive and Gram-negative strains, was investigated. The research included analysis of their action against both planktonic forms and bacterial biofilms. The compounds were tested for their antiadhesion properties on stainless steel and glass. It was found that the compound with a 16-carbon hydrophobic chain showed the greatest effectiveness against planktonic forms, as well as biofilms. QAS with 16 carbon atoms in the aliphatic chain also exhibited better antiadhesion activity to stainless steel surface than compounds with shorter chains. The compounds tested at MIC concentrations did not cause hemolysis of sheep blood cells. Compounds with longer aliphatic chains seem to have greater activity, with relatively low toxicity. None of the QAS tested showed mutagenic properties.

P016 Comparative assessment of bacteriophage, lactoferrin and antibiotic activity against multidrug-resistant *Staphylococcus aureus* biofilms

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Many scientists in the last decades have focused their research on designing novel biofilm treatment strategies, as the attached embedded-in-extracellular-polysaccharide-matrix bacterial cells exhibit increasing resistance to different disinfectants and antimicrobial agents. Bacteriophages are shown to be able to penetrate the inner layers of the biofilm and infect and lyse bacterial cells. Moreover, thanks to the mechanism of antibacterial action being completely different from that of antibiotics, phages are able to lyse multidrug-resistant bacterial strains. Alternatively, to enhance the activity, bacteriophages can be combined with other antibacterial agent.
In this study we tested the staphylococcal phages vB_SauM-A, vB_SauM-C, vB_SauM-D and an antimicrobial protein – lactoferrin against a biofilm formed by multi-drug resistant strains of *Staphylococcus aureus* and compared their effectiveness with antibiotics in biofilm eradication. CFU analysis showed a considerable reduction in staphylococci count in all bacteriophages used compared to control and to the antibiotic and lactoferrin treatment group (except the use of vB_SauM-A at strain 370). Concerning CV analysis of biofilm biomass, only strains 124 and 370 showed a statistically significant biofilm biomass reduction after antibiotics application. This reduction was similar to reduction caused by bacteriophages and lactoferrin.

P017 Defining the set of essential genes of invasive *Streptococcus anginosus* strain

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Streptococcus anginosus together with *Streptococcus constellatus* and *Streptococcus intermedius* form the *Streptococcus anginosus* group (SAG). For a long time, all SAG species were considered human commensals commonly found on the mucous membranes of the oral cavity, but also of gastrointestinal upper respiratory and urogenital tracts. However, in recent years, there has been a lot of evidence that SAG representatives have been associated with health problems such as abscess formation, lung infections and sepsis. The virulence mechanisms of the mentioned bacteria are largely unidentified. The aim of the study was to determine the set of genes necessary for *S. anginosus* under optimal conditions and to compare it with data for other human pathogens: *Streptococcus pyogenes* and *Streptococcus agalactiae*. A library of insertion mutants of the invasive strain *S. anginosus* 980/01 was constructed. The plasmid pGh9: IS*S1* was chosen as a donor of the IS*S1* insertion sequence; under appropriate conditions IS*S1* integrates into the bacterial genome at random locations. Deep sequencing of IS*S1* integration sites in the TraDIS strategy (transposon directed insertion-site sequencing) was performed on DNA

isolated from the library grown in a brain heart infusion (BHI) medium. In *S. anginosus* 980/01 strain 17% genes of 1825 predicted, are considered to be essential. Ca. 54% of essential genes are common to the three species. Additionally, of essential *S. anginosus* genes 6% are common to *S. pyogenes* only, and 8% to *S. agalactiae* only, and 18% are unique to *S. anginosus* 980/01.

Prevalence and susceptibility pattern of cerebrospinal fluid pathogens in patients in tertiary care of Kosovo

P018

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Acute bacterial meningitis is still considered one of the most dangerous infectious diseases. Identifying the pathogens and their susceptibility to antibiotics, and promptly using antibiotics is essential for treatment.
The aim of this study was to investigate the prevalence and antibiotic susceptibility pattern of cerebrospinal fluid pathogens in hospitalized patients.
The laboratory-based study was conducted during a 2 year period. Identification and antibiotic susceptibility testing of bacteria was performed by standard microbiological methods and Vitek 2 Compact. Results for susceptibility testing were interpreted based on EUCAST standard.
A total of 290 samples were cultured, causative bacteria were detected in 50 (17.2%). The majority of them were from a Neonatology Unit 34 (68%). Gram-positive bacteria and Gram-negative bacteria accounted for 52% and 48%, respectively. The most isolated strains were CoNS 17 (65.3%) and *Acinetobacter baumannii* 12 (50%), while other pathogens were detected in <5% of samples. Gram-positive bacteria displayed the highest sensitivity to vancomycin, linezolid and tigecycline 100%.
We found one isolate of vancomycin resistant *Enterococcus faecium*.
Acinetobacter baumannii was common in newborns (≤28 days) and all the isolates were multi-drug-resistant. There were no isolates of *Acinetobacter baumannii* with resistance to colistin. Only one isolate of *Candida albicans* was detected which was sensitive to all antimycotic drugs.
We comprehensively studied the pathogen characteristics and antimicrobial resistance patterns from samples of cerebrospinal fluid in our hospital, which can provide valuable strategies for preventing pathogens and improving evidence-based treatment.

P019 Survival of *Acinetobacter baumannii* in monocytes

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Acinetobacter baumannii is one of the critical opportunistic multidrug-resistant "priority" pathogens, classified as ESKAPEE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Escherichia coli*). It is the predominant etiological agent of ventilator-associated pneumonia, which is particularly dangerous for patients in intensive care units. In Poland, one in five patients develops such infections, which are associated with a high mortality rate. In addition to a large repertoire of resistance mechanisms, *A. baumannii* has the ability to effectively evade the host immune response. These rods release vesicles containing various virulence factors, including the porin protein OmpA, from their outer membrane and transport them into host cells. OmpA has been shown to play an important role in the colonisation of the lung epithelium by promoting adhesion, while inactivation of the *OmpA* gene results in a reduction of tissue damage. The aim of this study was to test the ability of selected strains (reference, clinical, mutant gene encoding LPS synthase and mutant gene encoding OmpA protein) to survive inside eukaryotic cells. After 2 hours of infection, monocyte line THP-1 cells were lysed with 1% triton and the lysate was spread onto LB plates at the appropriate dilution. After 24h, the CFU were counted. The results confirmed the ability of the selected strains to survive in THP-1 cells. The highest survival rate was recorded for the clinical strain. In contrast, the strain not producing OmpA was unable to survive in the cells. The experiment showed that *A. baumannii* is able to survive in THP-1 cells and that the OmpA protein is essential for this process.

P020 Antibiotic resistance genes and viral genomes assessment in wastewater samples from Cape Verde Wastewater Treatment Plants

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Cape Verde is an archipelago located in the Sahel region, susceptible to natural vulnerabilities like water scarcity, which cause extreme drought situations, leading the country to water calamity. Consequently, water crisis mitigation is crucial, and treated wastewater reuse is a sustainable and recommendable alternative, especially in agriculture irrigation. However, the quality of the water must comply with physico-chemical and microbiological requirements so that public health is not harmed.

The focus of this study was to evaluate the presence of antibiotic-resistant bacteria/genes (ARB/ARG) to carbapenems and fluoroquinolones, and of human pathogenic viral genomes in water from Wastewater Treatment Plants (WWTPs).
Influent and treated effluent of six WWTPs selected among Cabo Verde islands was collected and evaluated for presence of ARG and viral genomes by multiplex qPCR protocols. In parallel, bacteria resistant to these AB were isolated in a selective medium supplemented with meropenem or ciprofloxacin, and the AB resistance phenotypes, of the potentially pathogenic bacteria, assessed by the EUCAST disk diffusion method.
Polyomavirus, Adenovirus, Norovirus GII, Hepatitis A, and Hepatitis E genomes were detected at high concentrations (10^6 gc/l) either in the influent or effluent samples, as well as carbapenem and fluoroquinolones resistance genes, with concentrations of 10^{10} gc/l.
These results suggest that the conventional applied treatments in the studied WWTPs are not enough to remove ARG and viral genomes, which reinforces the need to develop/implement targeted treatments at full a-scale in the WWTPs, so that the produced effluents could be safely discharged into environment and/or reused for purposes like agricultural irrigation.

Overcoming current wastewater-based epidemiology limitations through combination of electrocharged granular materials and Flow Virometry

P021

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The SARS-CoV-2 pandemic has highlighted wastewater-based epidemiology (WBE) as an efficient and relatively accurate tool for community health monitoring purposes. Beyond SARS-CoV-2, WBE has a potential to be utilized for monitoring a broad range of virus-caused conditions. Gastroenteritis disease, for instance, is caused by pathogens including enteric viruses such as adenovirus, caliciviruses, enterovirus and hepatitis A virus. Those enteric viruses are known or anticipated to be present in public water systems. Virus contamination of water supplies is a public health concern because it can cause outbreaks or sporadic viral infections. However, due to several bottlenecks, the efficiency of WBE is restrained. Due to the small virus particles size and low abundance of these viruses in water, it is essential to concentrate large water volumes into small aliquots to enhance the probability of capturing a rare event. Membrane-based concentration methods depict the highest efficacy among equivalent methods available nowadays. However, high organic material load in wastewater can limit viral recovery. Therefore, a need for improved virus concentration technique exists. In this study, we tested the efficiency of commercially available and *de novo* synthesized electropositive and electronegative granular matrices and their combination to overcome the membrane clogging issue and potentially increase virome coverage. To ensure water safety we explored the combinatory use of flow cytometry (FCM) and qPCR to determine if FCM could be used to predict pathogenic virus concentrations present in water.

P022 What do human cells infected by the SARS-CoV-2 virus look like? FISH as an alternative method of detection and visualisation of viral infection

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In the diagnosis of SARS-CoV-2 virus infection, the reference method is qPCR. The application of other additional molecular methods with a broader detection range is desirable due to the constant mutation of the virus and for better epidemiological control.

The aim of the study was to develop a highly specific molecular test capable of detecting and visualizing SARS-CoV-2 infection.

The study included 290 patients with clinical signs of SARS-CoV-2 infection, from whom nasopharyngeal swabs were collected in double (n=580). One swab from the two collected was subjected to viral RNA isolation and amplification by qPCR. From the second swab, a microscopic preparation was performed using self-designed 18 oligonucleotides and fluorescence-labeled hybridization probe. The results were assessed under the BX63 Olympus fluorescence microscope in 3 channels: DAPI-blue, visualizing cell nuclei), FITC (green, visualizing the cell cytoplasm), and Texas Red-for detecting cellular SARS-CoV-2 replication areas.

In the case of the qPCR method, 200 results were positive, and 90 were negative, while the use of our fluorescent *in situ* hybridization (FISH) method allowed for 220 positive results (185 of which were accordance with the qPCR results) and 70 negative results.

The research conducted by our team supports the use of FISH as an additional method in the diagnosis of SARS-CoV-2.

The study was supported by the National Center for Research and Development CRACoV-HHS project (Model of multi-specialist hospital and non-hospital care for patients with SARS-CoV-2 infection) through the initiative “Support for specialist hospitals in fighting the spread of SARS-CoV-2 infection and in treating COVID-19”

Biological functions of *Staphylococcus aureus* Efb domains: potential therapeutic targets

P023

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Because antibiotic-resistant *Staphylococcus aureus* strains continue to proliferate, it is vital to investigate alternate targets for future therapeutic uses. As a result, it is critical to comprehend staphylococcal immune evasion mechanisms, with a particular emphasis on extracellular fibrinogen-binding protein (Efb) and Efb-related proteins. Efb includes three biologically relevant binding sites that might be employed as therapeutic. First, the fibrinogen-binding motifs present in coagulase inhibit neutrophil M2 adherence to fibrinogen and attract fibrinogen to the bacterial surface, where it forms capsule-like structures that inhibit phagocytosis. Second, Efb is a powerful anti-thrombotic agent, which is most likely due to its P-selectin binding ability. Efb P-selectin binding inhibits P-selectin interaction with the PSGL-1 receptor, impairing platelet-mediated leukocyte recruitment to the site of vascular damage. Third, the Efb complement binding domain, which is also present in other staphylococcal complement inhibitory proteins such as Ecb, Sbi, and SCIN, is responsible for complement-mediated immune response evasion. Efb inhibits the synthesis of C3 convertase and the contact with neutrophils, as well as the activation and maturation of B cells. Efb binding sites have a demonstrable impact on *Staphylococcus aureus* virulence in mastitis, wound infection, staphylococcal pneumonia, and infections due to implanted devices, and they contribute to staphylococcal persistence in host tissues and kidney abscess development. Given their biological importance in staphylococcal infections, Efb binding sites are interesting vaccine targets. Furthermore, because Efb inhibits platelets and complements, it may be a potential treatment for disorders linked with thrombosis and aberrant complement activity.

P024 *Clostridioides difficile* infection among COVID-19

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Despite the viral genesis of the Coronavirus disease, most clinicians began the use of oral or intravenous antibiotics at different stages. One of the main problems associated with long-term antibiotic abuse is *Clostridioides difficile* infection (CDI). We studied 136 patients with a mean age of 58 years (20–92 years) for a 17-month period, who suffered from COVID-19 and were subsequently diagnosed with CDI. Banatrol, a new generation food supplement is included in the main antimicrobial and symptomatic therapy. The 136 patients studied received their first symptoms of CDI on average 12.6 days after discharge from the COVID-19 units.

The symptoms were: diarrhoea syndrome (100%), abdominal pain (83.3%), and fever (75%). Some patients had leukocytosis (68.3%), less often with hypokalemia. All patients received prior antibiotic therapy – with one (34.6%), two (52%) or three antibiotics (13.4%). Patients with CDI were hospitalized for an average of 9.8 days, with 76.4% of them discharged with improvement, 13.4% had a relapse, and one patient died. In 60 randomly selected patients, banana peel fibre Banatrol was used along with classic diet. In these patients, the diarrhoea syndrome lasted on average 2.6 days less and we observed only 4 patients with relapse. Adult patients with severe concomitant pathology tend to develop severe CDI, with the time of hospitalization and the risk of recurrence largely dependent on the interval between recovery from the acute phase of COVID-19 and the onset of the first symptoms of intestinal infection.

This research was funded by grant no. BG05M2OP001-1.002-0001-C04.

Phagosomal acidification and autophagy are required to kill *Streptococcus pneumoniae* in a zebrafish model

P025

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Streptococcus pneumoniae is a major human pathogen causing invasive disease, including community-acquired bacteraemia, and remains a leading cause of global mortality. Understanding the role of phagocytes in killing bacteria is still limited, especially *in vivo*. Here, we established a zebrafish model to study interactions between intravenously-administered pneumococci and professional phagocytes (macrophages and neutrophils), to unravel bacterial killing mechanisms employed by these leukocytes. Our model confirmed the key role of polysaccharide capsule in promoting pneumococcal virulence through inhibition of phagocytosis. Conversely, we show pneumococci lacking a capsule are rapidly internalised by macrophages. Low doses of encapsulated *S. pneumoniae* cause near 100% mortality within 48 hours post-infection (hpi), while 50 times higher doses of unencapsulated pneumococci are easily cleared.
Timecourse analysis of *in vivo* bacterial numbers reveals that while encapsulated pneumococcus proliferates to levels exceeding 10^5 CFU at the time of host death, unencapsulated bacteria are unable to grow and are cleared within 20 hpi. Using genetically-induced macrophage depletion we confirmed an essential role for macrophages in bacterial clearance. Additionally, we show that upon phagocytosis by macrophages, phagosomes undergo rapid acidification. Genetic and chemical inhibition of vacuolar ATPase prevents intracellular bacterial killing and induces host death indicating a key role of phagosomal acidification in immunity to invading pneumococci. Preliminary experiments also revealed the involvement of autophagic response during degradation of pneumococci within macrophages. Collectively, our data confirm larval zebrafish can be used to dissect killing mechanisms during pneumococcal infection *in vivo* and highlight key roles for phagosomal acidification in macrophages for pathogen clearance.

P026 Exploring the virulence potential of *Prototheca* microalgae in murine model

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Prototheca spp. are unicellular, achlorophyllous algae, which are the only known plants capable of causing opportunistic infections (protothecosis) in both animals and humans. This work aimed to investigate the virulence potential of the *Prototheca* algae by assessing the ability to induce local or systemic infections in an experimental murine model.
Type strains of three pathogenic (*P. wickerhamii*, *P. bovis*, and *P. ciferrii*) and one non-pathogenic (*P. stagnora*) species were used to experimentally infect immunocompetent and immunodeficient mice. The study was carried out on 54 groups (6 individuals per each) depending on the inoculum (algae or PBS as a control), the challenging dose (i.e. $5x10^6$ or $5x10^7$CFU/ml), and inoculation route (subcutaneous, intramammary and intraperitoneal). The infection doses were applied to 10-week, female mice. Six weeks post-infection, the mice were euthanized, and their organs were explanted, weighted, homogenized with TissueLyser (Qiagen, Germany), and subjected to serial dilutions, subsequently plated on Sabouraud agar and incubated for 72 hours at 30°C.
In this study 288 animals in 48 groups (2x24 groups of mice of each strain) were infected with *Prototheca* sp. Twenty-eight (19.4%) wild type mice and almost twice that (37.5%) immunodeficient animals showed signs of infection. *P. ciferrii* accounted for the majority of infections (45.1% of all cases). This was followed by *P. bovis* (34.1%) and *P. wickerhamii* (20.7%). It thus appears that *P. ciferrii* has the highest virulence capacity. Mice with impaired immune system are more prone to develop *Prototheca* infection (28/144 vs 54/144; 18.1% more cases; $p < 0.05$).

P027 Real-time PCR for quantitation of bacteria – new approach

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Through 40 years of existence real-time PCR has been highly developed. Now, it is affordable and easy to perform. Modifications such as digital droplet PCR and others have been successfully introduced. Microbiology scientists saw the opportunity in the use of real-time PCR for enumeration of bacterial cells. This may help in diagnosing bacterial infections caused by biofilm. Since 2000's numerous original articles have been published. However, there is no diagnostic tool to enumerate bacterial cells in samples.
Five *Proteus mirabilis* strains were used in the experiment. Real-time PCR was utilized. Fluorescence was measured and curves were analyzed. Simultaneously, the number of bacterial cells was measured using the plate count method.

As a result, each *P. mirabilis* strain was characterized by different standard curve and R^2 was between 0.88 and 0.92. However, when combined, the standard curve for 5 *P. mirabilis* strains had R^2 of 0.78. This leads to the conclusion that a combined curve is not as useful and each examined strain should have its own standard curve developed.
Unfortunately, out of 55 studies, many skip this stage of standard curve developing. This may be the reason for difficulties in experiment recreation in other laboratories.
The main postulate of this work and the analysis of the available studies is –real-time PCR for the enumeration of bacterial cells should be used with caution. Standard curves should not be only developed using molecular constructs or pure nucleic acids. *In vitro* tests on bacterial cells in models mimicking the *in vivo* environment are necessary.

Varying levels of adhesion to fibronectin and fibrinogen by *Staphylococcus aureus* bacteremia isolates – the role of genetic variance and associated clinical manifestations

P028

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Staphylococcus aureus is one of the most common sources of infections in the soft tissues and blood which can cause life-threatening complications such as infective endocarditis. This study focused on the importance of microbial surface components recognizing Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) that are responsible for binding cells to human glycoproteins: fibrinogen (ClfA & ClfB) and fibronectin (FnBPA & FnBPB). The aims of the project included identification of genetic variance associated with distinctive levels of adhesion and investigation of the role of adhesion in the development of bacteremia and its complications.
A newly assembled collection of 250 *Staphylococcus aureus* bacteremia isolates from three hospitals in Poland were subjected to whole genome sequencing and phenotyped quantitatively to discriminate their ability to adhere to fibrinogen and fibronectin. In the case of fibrinogen, the cultures were carried out to the stationary phase, for fibronectin – to the log phase.
Investigated bacteremia isolates represent varying levels of adhesion. The distribution of adhesion levels was normal. The discriminatory power of the data is sufficient identification of associated genetic variance and clinical manifestations. Initial analysis showed significant differences in the level of adhesion to fibronectin between strains derived from different sequence types and clonal complexes. Moreover, community-acquired isolates had higher levels of adhesion to fibrinogen than the healthcare-associated strains while mortality of patients infected with more adhesive isolates was lower.
The ongoing genome-wide association studies will identify the genetic variance responsible for the varying levels of adhesion linking the genotype with clinical manifestations of the patients.

P029 Drug resistance assessment tools evaluating positively selected amino acid sites in proteins of *Mycobacterium tuberculosis*

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Amino acid sites with increased quantity of nonsynonymous substitutions observable across the population show signatures of positive selection. In bacteria living under antibiotic pressure, positive selection might signal sites involved in the generation of drug resistance. We estimated the fraction of positively selected sites possibly generated through drug resistance in drug target proteins in the model of the bacterium *Mycobacterium tuberculosis*. We calculated the level of selection per each amino acid site of twenty drug-resistance-associated proteins of 3978 clinical strains of *M. tuberculosis*, and we compared our list with two bioinformatic databases commonly used to establish drug resistance – TB Profiler and MTBSeq. We found that both programs labeled roughly 40% of positively selected sites as associated with drug resistance. We conclude that the generation of drug resistance explains the presence of at least one-third of amino acid positions under positive selection in *M. tuberculosis* protein drug targets. We suggest that the inclusion of observation of evolutionary patterns might facilitate prioritizing particular mutations for further research and observation, eventually leading to better drug resistance diagnosis, better treatment outcomes, and the reduction of costs to control tuberculosis.

P030 Further insights into the genomic panorama of the *Mycobacterium kansasii* complex

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The *Mycobacterium kansasii* complex (MkanC) is composed of the closely related species *M. kansasii*, *M. persicum*, *M. pseudokansasii*, *M. ostraviense*, *M. innocens*, and *M. attenuatum*, and *M. gastri*. Some MkanC species are frequently isolated from humans and reported as colonizing, opportunistic or pathogenic and cause tuberculosis-like lung or extrapulmonary diseases. Moreover, different strains of the MkanC can present heterogeneity regarding phenotypic traits such as colony morphology and biochemical traits, drug resistance, virulence, and pathogenicity. We performed comparative genomics and phylogenomic analyses on a collection of 665 MkanC genomes – 342 having been sequenced in this study – and the majority being *M. kansasii* and *M. persicum*. The isolates were from 29 countries and included 27 from Brazil; the data on drug resistance, morphology and virulence were (partly) available. Our data led to a better insight of the MkanC global distribution, and we observed a surprisingly high frequency of signatures for mobile genetic elements such as plasmids (37%) and prophages (80% of the genomes). We also observed the presence of 17 distinct antiviral defense systems. Despite the high number of genomes, the MkanC pangenome remains open, with a highly diverse accessory genome. We also propose a putative MkanC virulome, defining the presence/absence of species-specific virulence factors. A Bayesian inference on the *M. kansasii* phylogenetic tree allowed reconstruction of an ancestral scenario, inferring the emergence of recent *M. kansasii* variants. Finally, we have defined almost 300 structural variants within *M. kansasii* and observed lineage-specific deletions ranging from ~ 0.2 to over 66 kbp.

P031 Characterization of *Pseudomonas aeruginosa* isolated from burn patients in Uruguay

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Pseudomonas aeruginosa is a main pathogen in burn patients. Biofilm production is a pivotal pathogenic mechanism involved in chronic infections.
Aim: To describe *P. aeruginosa* isolates from patients admitted to the National Burn Center in Uruguay (CE.NA.QUE): phenotypic characteristics, antibiotic resistance, ability to produce biofilm.
37 isolates from 23 patients from different body sites were included. Antibiotic susceptibility was studied with VITEK2®. Strains were grown on tryptic soy agar to observe mucoidity and pigment production. Biofilm production was studied by quantitative crystal violet stain. The study was approved by the directive board from CE.NA.QUE.
73% of patients were male; the mean age was 56 years; the mean percentage of total body surface area (TBSA) was 26%. 68% of isolates were from burn wounds, 32% from other sites; 62% exhibited mucoid phonotype; 54% were non-pigmented. Non-susceptibility (NS: resistant + intermediate) rates were: tazobactam-piperacillin (65%), meropenem (62%) and imipenem (57%), gentamicin (49%), ciprofloxacin (46%), amikacin (24%), ceftazidime (24%). 32% were susceptible to all antibiotics, 58% were multi-resistant (NS to 3 different antibiotic classes). 36/37 strains were biofilm producers. Biofilm strong producers were more likely to be amikacin, gentamicin, ciprofloxacin or tazobactam-piperacillin NS ($p < 0.05$). All isolates from burn wounds were strong biofilm producers while none of the isolates from other sites were strong producers (p 0.0022). The isolates exhibited high resistance to beta-lactams. Most strains were biofilm producers. There is a correlation between some morphotypes, isolate origin and NS to some antibiotics. Molecular studies are being conducted to further understand the behavior of *P. aeruginosa* in these patients.

Is it possible for future microbiology diagnostics to belong to artificial intelligence?

P032

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Current trends in clinical laboratories are headed towards the simplification, automatization, cost and time reduction of standard diagnostics procedures. In routine microbiological diagnostics, the pathogenic microorganism identification process consists of many steps and may take from a few days to several weeks. Other alternative methods e.g., PCR or MALDI-TOF mass spectrometry have higher sensitivity compared to culture methods, but due to the cost of equipment and/or reagents and qualified laboratory technician, they are not yet widely used.
The aim of the study was to assess the suitability of the use of artificial intelligence to identification of interspecies bacterial mixes in Gram-stained preparation images.
The analysis embraced 4 representative species of Gram-positive (*Staphylococcus aureus* and *Lactobacillus plantarum*) and Gram-negative (*Escherichia coli* and *Neisseria gonorrhoeae)* bacteria. Gram-stained preparations were prepared in duplicate from the obtained reference strains and from their 11 interspecies mixes. Then, from each preparation 20 images were taken with the use of BX63 microscope (Olympus). The images were divided into smaller fragments with the use of deep bag of visual words method. All fragments were passed through deep neural network convolution block and aggregated using a Fisher vector. Finally, they were subjected to the classification phase using the SVM method.
The conducted experiments resulted in a high degree of distinguishability of single species in images obtained from multi-species mixtures (ROC AUC 0.972). The performed studies resulted in a patent EP22461550.0.
The research results open the way for further development and refinement of the algorithm created by our team for the diagnostics of clinical materials.

Exit from Krakowskie Przedmieście Street towards Castle Square, Warsaw, 1930s.

Photo by Henryk Poddębski, The Museum of Warsaw.

II. A-nimals

Microbial Diseases of Wild and Domesticated Animals: Aetiology – Epidemiology – Diagnostics – Treatment

Zoonotic *Acinetobacter baumannii*: exploratory epidemiological investigation towards "One Health" approach

P033

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Until 2000's *Acinetobacter* (*A.*) *baumannii* infections were rare and were readily treated. Currently, *A. baumannii* accounts for 600,000 to 1,400,000 infections globally per year and is one of the six leading nosocomial infections. The rates of this nosocomial pathogen, already known for its resistance against carbapenems, are reported to have increased in 2020. The clinical samples isolated from animals and humans share identical clones, suggesting that the animals may act as reservoirs. However, the data from animal origin, especially from the healthier ones, are rarely available to establish *A. baumannii* interplay between environment, animals, and humans. And there is no study reported on these aspects from India. Therefore, an exploratory research study was undertaken to investigate the distribution of *A. baumannii* among healthier animals (large ruminants, small ruminants, and poultry) in the rural region of Guntur, India. Samples representing five different systems (integumentary, digestive, respiratory, excretory, and urogenital system) resulted in 51 non-duplicated isolates, which is 49% prevalence. No isolates were obtained from the salivary samples. The species identification was carried out using microbiological analysis and MALDI TOF MS. The Kirby Bauer disc diffusion assay, using 16 antibiotics representing eight different classes, indicated that these isolates except those isolated from excretory system (n = 10, ~ 20%) possess comparable antimicrobial profiles: resistant to penicillin, aminoglycoside, cephalosporin and lincosamide, intermediate resistance to fluoroquinolone, meropenem (carbapenem) and vancomycin (glycopeptide) and susceptible to tetracycline. As far as our knowledge is concerned, this is the first study of its kind from India.

P034 Staphylococci isolated from Polish primitive sheep breeds – superantigen toxin gene profile and antimicrobial resistance

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The study aimed to analyze staphylococcal microbiota of the nasal cavity of the primitive native sheep breeds Polish Świniarka and Wrzosówka kept on the same ecological and agritourism farm. The research included the identification of bacterial species, evaluation of the prevalence of genes encoding enterotoxins, staphylococcal enterotoxin-like proteins, exfoliative toxins, toxic shock syndrome toxin 1, and detection of antimicrobial resistance. From 61 swab samples gathered from healthy Świniarka (33) and Wrzosówka (28) sheep, 127 coagulase-negative staphylococci (CoNS) were isolated. Based on PCR-RFLP analysis of the gap gene using *Alu*I and *Hpy*CH4V enzymes, the isolates were identified as: *Staphylococcus xylosus* (33.9%), *S. equorum* (29.1%), *S. arlettae* (15%), *S. warneri* (9.4%), *S. lentus* (7.9%), *S. succinus* (3.9%) and *S. sciuri* (0.8%). Three of these species, *S. lentus*, *S. succinus*, and *S. sciuri*, were detected only from the Świniarka breed. It was found that 77.2% of isolates harbored from 1 to 7 out of 21 analyzed genes for superantigenic toxins. The greatest diversity of toxin genes was recorded for *S. equorum* (16 different genes). The most prevalent gene was *ser* (40.2%). The incidence and number of resistances to antimicrobials were found to be bacterial species but not sheep breed dependent. The highest percentage of resistance was found for *S. sciuri*. The most frequent resistance was observed to clindamycin (45.7%). The findings of this study seem to be essential to provide background information on the incidence, species diversity, and potential pathogenicity of commensal staphylococcal biota colonizing the nasal cavity of healthy sheep.

Adhesive potential of lactic acid bacteria (LAB) selected for anti-*Campylobacter* prophylaxis

P035

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Campylobacter sp. is the most commonly isolated human enteropathogen. Consumption of contaminated poultry meat is the main source of disease. One of the proposed strategies to prevent human infections is poultry vaccination. In the Department of Bacterial Genetics, UW we are assessing the application of lactic acid bacteria (LAB) probiotic strains carrying *Campylobacter* antigens as a vaccine booster.

One of the mechanisms that probiotic bacteria have developed to resist the peristaltic movement of the intestines is the ability to adhere to epithelium or mucus. This prolongs the persistence of bacteria in this specific environment and is an important feature for probiotic strain selection. Therefore, we investigated the adhesive potential of a preliminarily characterised LAB strain collection isolated from backyard chicken flocs. First, twelve selected strains were examined for their ability to adhere to LMH cells. Next, we screened their genomes and plasmids for genes encoding potential adhesins – large molecular weight proteins (above 220 kDa), containing numerous amino acid repeats, which is a landmark of SRRPs (serine-rich repeat proteins). Recently, these proteins have been proposed as significant elements of bacterial attachment to the intestinal epithelium. Finally, several strains of *Lactiplantibacillus plantarum* were checked for *in vivo* chicken colonization.

In silico analysis did not reveal any SRRPs in the selected proteomes. However, we identified various other potential adhesins with specific amino acid repeats that will be further investigated. Out of four LAB strains examined *in vivo*, *L. plantarum* 18A showed the longest persistence in chicken intestines and will be used as an anti-*Campylobacter* antigen carrier in vaccine booster for chickens.

This research was funded by National Science Center, Poland (2016/21/B/NZ6/01141).

The Bank of National Economy, Warsaw, 1933.

Photo by Henryk Poddębski, The Museum of Warsaw

III. S-ystems

Biofilm – Microbiome – Genomics, Metagenomics & Other –Omics

Analysis of oral microbiota after infection with SARS-CoV-2 P036

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According to the latest literature data, SARS-CoV-2 infection may have a direct influence on changes in bacterial profiles of the oral cavity.

The aim of the study was the qualitative and semi-quantitative assessment of the composition of the oral microbiota of the patients after SARS-CoV-2 infection and hospital treatment.

The study included 60 participants: 40 patients after hospitalization in a university hospital due to SARS-CoV-2 infection (research group) and 20 healthy volunteers (control group). The biological materials included: saliva, swab from the back of the tongue, supragingival and subgingival plaque. From the collected materials, bacterial DNA was isolated, which served as a template for the amplification of the regions V3 and V4 by PCR. PCR products were used to prepare the library for Next Generation Sequencing (NGS).

Alpha and beta biodiversity in all patients and all biological materials were statistically significant at both the phylum and species levels ($p<0.004$). Core microbiome for all studied material consisted mainly of: *Veillonella dispar* and *Prevotella melaninogenica*. Statistically significant differences were found in the composition of the microbiota in each of the tested materials between the studied and control groups, ranging from 24 to 101 species. Based on the LEfSE analysis, marker bacteria were determined e.g., in saliva, and the studied group differed from the control group by the absence of *Faecalibaculum rodentium* and *Akkermansia muciniphila*.

The use of NGS allowed to show the differences in the composition of the oral bacterial microbiota between patients after SARS-CoV-2 infection and healthy people.

P037 Enhancement of inhibition of *Pseudomonas* sp., biofilm formation on bacterial cellulose-based wound dressing by combined action of alginate lyase and antibiotics

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Pseudomonas aeruginosa is an opportunistic pathogen that causes nosocomial infections whose treatment with standard antibiotics is often unsatisfactory due to the ability of these bacteria to form a biofilm. A widely used method for removing biofilm from a chronic wound and improving its healing process is the mechanical removal of necrotic tissue and microorganisms, followed by antibiotic therapy. However, infections that are caused by bacteria that can form a biofilm require longer treatment with antibiotics, which sometimes is completely ineffective. Moreover, the extended time of healing and prolonged use of antibiotic can lead to many dangerous secondary effects for patients. Bacterial cellulose (BC) is a promising dressing material with high stability, biocompatibility, and high water holding capacity. For wider field of application in wound healing, BC can be saturated with various chemical compounds such as proteins and enzymes. The aim of this study was the analysis of recombinant alginate lyase (AlgL) as a factor for improving BC dressing safety. First, due to its use as a wound dressing, the influence of AlgL on cytotoxicity towards eukaryotic cells was tested. Next, AlgL was immobilized on BC membranes via physical adsorption and the effect of immobilization on the degree of reduction of biofilm produced on the BC surface by *P. aeruginosa* was assessed. In addition, the effect of the simultaneous immobilization of AlgL and gentamicin on the viability of bacterial cells was investigated. The obtained results showed that the use of AlgL allows for reduction of gentamicin dosage being necessary for significant *P. aeruginosa* biofilm development inhibition.

Effects of tyrosol and farnesol combinations with antimicrobial agents on *Candida albicans* and *Pseudomonas aeruginosa* single and dual-species biofilms

P038

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Candida albicans is commonly found with *Pseudomonas aeruginosa* in dual-species biofilms, especially in cystic fibrosis patients. Because dual-species biofilms are more complicated and difficult to eradicate than single-species biofilms, antimicrobial selection for infections caused by dual-species biofilms presents additional challenges for treatment. The aim of this study was to examine the effects of *C. albicans* quorum sensing compounds, tyrosol and farnesol, combination with antimicrobial agents on *C. albicans* SC5314 and *P. aeruginosa* PA01 single and dual-species biofilms in 96-well tissue culture-treated polystyrene plate.

Farnesol (300 µM), tyrosol (80 µM), fluconazole (10 µg/ml), amphotericin B (4 µg/ml), caspofungin (2 µg/ml), aztreonam (500 µg/ml), colistin (200 µg/ml) and tobramycin (200 µg/ml) assessed against biofilms and viability was monitored by CFU assay *in vitro* and in the *Caenorhabditis elegans* infection model.

Our results demonstrated that farnesol possesses an antibiofilm activity against microorganisms greater than tyrosol. We observed that farnesol combination with antibiotics, especially with colistin, were more effective against *P. aeruginosa* in single and dual species biofilms, than tyrosol combinations. Nevertheless, tyrosol combination with caspofungin exerted strong effects against *C. albicans* in single and dual species biofilms. Increased survival was observed for *C. elegans* when treated with colistin in combination with farnesol or tyrosol, compared to no treatment or other antimicrobials.

Quorum sensing molecules especially in combination with antimicrobial agents can exert an antibiofilm effect. This can be considered as an alternative for the eradication of dual species biofilms in particular, which are very difficult to treat.

P039 Virulence differences of *Staphylococcus aureus* nasal carriage in patients after breast cancer qualificated to breast reconstruction

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Staphylococcus aureus are the major etiological agents of breast peri-implant infections. It is of great significance to carry out the monitoring of the prevalence and virulence of *S. aureus* strains from nasal carriage in the qualification of patients for breast reconstruction in the context of potential *S. aureus* infections.

The retrospective study was carried out on a group of 33 *S. aureus* strains isolated from nasal carriage of 32 patients who underwent breast reconstructive at the Oncology Center – prof. Franciszek Łukaszczyk Memorial Hospital in Bydgoszcz. In the study the susceptibility of *S. aureus* to antibiotics was analyzed. Slime production was obtained on CongoRed agar. Biofilm formation was evaluated with the use of the colorimetric assay and quantitative CFU/ml determination. The genotypic characterization included detection of genes important in extracellular polysaccharides synthesis and intracellular adhesion (*icaA*, *icaB*, *icaC*, *icaD*), and the genes that play role in surface adhesion (*cna*, *sasG*).

Two *S. aureus* strains were resistant to erythromycin/clindamycin or to tobramycin. All isolates presented MSSA phenotypes. 97% of the total isolates were strong biofilm producers. Slime production for 79% isolates was detected. Virulence genes *icaA*, *icaB*, *icaD*, cna and *sasG* were present in all strains. 15% were *S. aureus* with an *icaC* gene deletion.

In the study of *S. aureus* from nasal carriage from patients who underwent breast reconstructive important virulence factors were detected. The research should be continued in order to detect the relationship between virulence factors and the presence of *S. aureus* nasal carriage infection in patients after breast cancer.

Topology of *Pseudomonas aeruginosa* chromosome: the role of partitioning system ParAB-parS

P040

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In the majority of bacterial species, including the pathogen *Pseudomonas aeruginosa*, the tripartite ParAB-*parS* system, composed of an ATPase (ParA), a DNA-binding protein (ParB), and its target *parS* sequence(s), facilitates the initial steps of chromosome partitioning. ParB forms large nucleoprotein complexes at *parS*(s), palindromic sequences located in the vicinity of *oriC*, which after replication are subsequently relocated by ParA to polar positions. Remarkably, our recent ChIP-seq analysis showed that ParB of *P. aeruginosa* binds not only to palindromic *parS* sites but also to numerous secondary binding sites containing one arm of the *parS* palindrome (half-*parS*). Here, we analyzed the role of ParB binding to half-*parS*s in maintenance of the topology of bacterial chromosome. Using chromosome conformation capture (3C-seq) we discovered features of the *P. aeruginosa* chromosome which included (i) the presence of chromosome interaction domains, (ii) contacts between opposing arms of the circular chromosome, mediated by Smc, and (iii) extensive interactions between *oriC*-proximal regions with various loci along the chromosome. The functional ParAB-*parS* was required both for Smc loading and for *oriC* region interactions. The ParB-*oriC* nucleoprotein complex longitudinal interactions with chromosome arms are promoted by ParB binding to half-*parS* sites. Moreover, by expanded ChIP-seq analysis, we showed that the half-*parS* sequences are universal targets for ParB binding under various growth conditions. Overall, these analyses demonstrate a multilayered role of partitioning proteins in the organization of the bacterial chromosome. This work was supported by the National Science Centre in Poland (grant no. 2018/29/B/NZ2/01745).

P041 Laboratory sewage settling tank metabiome

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We aimed to characterize the microbial community of sewage sludge collected from a sedimentation tank processing laboratory wastewater from the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences (IBB PAS). At some point during its use, the flow of wastewater was practically stopped. We suspected that these problems may have been due to the nature of the unique microbiota developing in the tank.

Samples from four different tank spots were collected. Microbial diversity was examined using both culture-dependent and -independent techniques. Almost 200 fungal and bacterial strains with different colony morphology were isolated and preserved. For an in-depth analysis of the tank microbiome, we performed whole population metagenomic sequencing using Illumina and Oxford Nanopore techniques (>164 GB). Preliminary results indicate high biodiversity and significant differences in the proportions of the occurrence of individual phyla. Representatives of *Bacillus* and *Pseudomonas* genera dominated among cultivated strains. We also did a preliminary assessment of microbiome metabolic potential related to carbon substrates and we found that this potential was the highest in the first two stages of wastewater filtration. We will study the presence of mobile elements, heavy metal, and antibiotic resistance genes, but also other genes conditioning resistance of this microbiome to substances present in the laboratory wastewater.

The results of the project will be the first full characterization of microbial communities selected under the conditions of long-term collection of sewage in biological and chemical laboratories.

The work is supported by a microgrant from the IBB PAS, no.: DEC-MG-3/22-01.

Additional *parS* sites are essential components of partitioning systems of *repABC* replicons in *Alphaproteobacteria*

P042

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DNA segregation during cell division is one of the basic biological processes ensuring stable inheritance of genetic information. In bacteria, partitioning systems enable maintenance of low-copy number replicons (e.g. chromosomes, chromids and megaplasmids). Partitioning systems are usually encoded by a separate functional cassette. The *repABC* modules are an exception. They constitute a unique structural and regulatory coupling of genes determining two important functions: DNA replication and partitioning. In *repABC* replicons, the partition (*repAB*) and replication (*repC*) genes form a single operon, with *parS* sequences usually positioned in close proximity to these genes.

Genomic analysis of *Paracoccus aminophilus* plasmid pAMI4 (438 kb) revealed that this replicon, besides the *repABC* operon (with *parS* located between *repB* and *repC*), contains a non-coding locus, 12 kb downstream of *repC*, carrying three additional *parS* repeats (*3parS* locus). The main aim of our study was to verify whether the *3parS* locus is involved in pAMI4 stabilization.

We have demonstrated that *3parS* (a) exerts incompatibility towards the parental replicon, (b) is bound by the RepB protein *in vitro*, and (c) is essential for correct pAMI4 partitioning *in vivo*. Analysis of *parS* distribution in sequenced *repABC* replicons identified other unrelated distantly placed *parS* loci, which had previously been overlooked. Some of these *parSs* are located within *cas* genes of CRISPR-Cas system. Several loci closely resembling the *3parS* of pAMI4 were identified in *Allorhizobium vitis* S4 chromosome 2 and related replicons. The obtained results shed new light on the *repABC* replicon structure and functioning.

P043 Distribution of individual *Candida* species depends on Crohn's disease activity index

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Emerging data suggest that fungi colonizing the gastrointestinal tract may be associated with the etiology or activity of Crohn's disease (CD). Numerous studies point to fungi of the genus *Candida* as one of the main microbiological factors in this disease. However, the specific species could not be identified.

The aim of our study was to determine the share of individual species of *Candida*, depending on the pediatric disease activity index (PCDAI).

Material subjected to the analysis embraced stool samples collected from pediatric patients with: (I) active CD (n = 66). In this group, two subgroups were distinguished; (Ia): patients newly diagnosed (n = 50); (Ib): patients previously diagnosed and treated (n = 16). (II) non-active CD (n = 38); (III) – control group of healthy children (n = 39). Fungal DNA was isolated from the samples. Next, NGS sequencing was performed (MiSeq-Illumina).

The dominating species in group Ia were: *C. albicans* (22% of total species), *C. tropicalis* (10%), *C. dubliniensis* (9%). Group Ib was the most colonized by: *C. dubliniensis* (19%), *C. zeylanoides* (12%), *C. albicans* (11%). In turn, *C. albicans* (32%), *C. dubliniensis* (6%) constituted the richest community in group II. In group III, only 17% of *C. albicans* and 4% of *C. dubliniensis* were documented.

Positive correlation of *C. dubliniensis* with calprotectin ($p < 0.003$, $r = 0.29$), *C. sake* with calprotectin ($p < 0.04$; $r = 0.19$) and *C. tropicalis* with PCDAI ($p < 0.006$) was observed.

The distribution of individual *Candida* species varies depending on the activity of CD. Perhaps some species may be involved in maintaining exacerbation of the disease and in the future may constitute a predictor of symptoms.

This research was funded by National Science Center, Poland (2019/33/N/NZ5/00698).

Exoproteomes of *Porphyromonas gingivalis* type IX secretion system mutants P044

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Type IX secretion system (T9SS) of oral pathogen *Porphyromonas gingivalis* is responsible for translocation of secreted proteins, including numerous virulence factors, across the outer membrane. The secreted proteins are proteolytically modified and immobilized on the bacterial cell surface via an attached anionic-LPS. They can be also released into the extracellular environment embedded in outer membrane vesicles (OMVs). Mutations in genes encoding structural or functional components of T9SS lead to the retention of unprocessed, enzymatically inactive gingipains (major *P. gingivalis* virulence factors) in the periplasm, which results in attenuation of *P. gingivalis* pathogenicity. Based on the lack of gingipain activity in cultures of *P. gingivalis* it was assumed that T9SS mutants accumulate all T9SS cargo proteins in the periplasm. Interestingly, this accumulation of secreted proteins in the periplasm was variable for different cargos and exerted no apparent negative effect on *in vitro* growth and *in vivo* fitness of *P. gingivalis*. This is in odds with the periplasm capacity, which is limited and aggregation of proteins in this compartment usually triggers a degradation pathway. Here we explain this phenomenon by showing that most T9SS mutants have the “leaky” phenotype and release large amounts of cargo proteins into the culture medium. Of note, the T9SS cargo proteins identified in the medium were proteolytically unprocessed and lacked only the signal peptide cleaved off during the protein export into the periplasm via the Sec system. Moreover, the released gingipains occurred in zymogenic forms indicating that progingipains activation is also dependent on functional T9SS.

P045 Genome wide dissection of secondary chromosome initiation network in *Vibrio cholerae*

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Contrary to the vast majority of bacteria that possesses a unique chromosome, *Vibrio cholerae*, the pathogenic agent responsible for cholera disease, contains a genome divided into two replicons: a main chromosome and a secondary chromosome. Secondary chromosomes appear independently in many bacterial phyla and are often associated with pathogenic or symbiotic traits. Secondary chromosomes result from the acquisition and domestication of large plasmids. Indeed, some "megaplasmids" have cohabited with their host over a long evolutionary period and have become an obligatory component of their host's genome, essential for their normal metabolism. During their domestication, secondary chromosomes acquired different features allowing their maintenance through cell divisions and ensuring their vertical transfer to subsequent generations. The study of multipartite genome bacteria allows questioning the selective benefit of an organism to possess several chromosomes. In addition, how can a horizontally acquired genetic element connect to a pre-established genetic network?
One of the major steps in megaplasmid domestication is their integration into the host cell cycle. In *V. cholerae*, Chr2 replication is coordinated with that of Chr1. Chr1, which is 3 times larger than Chr2, is initiated first and when 2/3 has replicated, an initiation signal triggers the replication of Chr2 so that both chromosomes terminate replicating concomitantly. This initiation signal is emitted when the replication fork passes through a non-coding Chr1 locus named crtS (Chr2 Replication Triggering Site). Our work aims to elucidate the mechanism of this novel bacterial replicative checkpoint. In order to dissect this initiation network, we used high-resolution ChIPseq analysis.

Enhanced multiplication of *Listeria monocytogenes* in the presence of xanthohumol

P046

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Xanthohumol (XH) is obtained from hop (*Humulus lupulus* L.) by Supercritical Fluid Extraction with CO_2 as a solvent, at pressures ≥100 MPa. It exhibits strong antimicrobial activity mostly against Gram-positive bacteria. It was recently reported that the addition of hop extract to meat products, especially sausages, may prevent the multiplication of *L. monocytogenes*. Hop extracts contain a variable amount of XH ranging between < 1 and > 80%, which is why XH can hypothetically be found in sub-MIC concentrations in the meat product preserved with hop extract. In this study, the influence of sub-MIC XH concentrations from 10 µg/ml (1/2 × MIC) to 0.009 µg/ml (1/2056 × MIC) on the formation of *L. monocytogenes* biofilms was investigated. Sub-MIC concentrations between 1/8xMIC (1.25 µg/ml) to 1/2056 × MIC (0.009 µg/ml) stimulated biofilm production and increase in biomass by 13.5% to 59% of biofilms of 3 clinical *L. monocytogenes* strains. The influence of XH on pre-formed *L. monocytogenes* biofilms was investigated with 20 µg/ml (1 × MIC), 40 µg/ml (2 × MIC), 80 µg/ml (4 × MIC), and 160 µg/ml (8 × MIC) concentrations. All concentrations caused decreasing in biofilm biomass but increased multiplication of viable cells (CFU/ml) within the biomass (1–2.5 log (CFU) increase) of *L. monocytogenes* isolated from a dead sheep and in a strain isolated from frozen salmon. As a conclusion, depending on the concentration and bacterial growth stage, xanthohumol can exert both an antibacterial and stimulating effect on the multiplication and biofilm formation by *L. monocytogenes*.
(The research was carried out within Opus 18 Grant UMO-2019/35/B/NZ9/02774).

P047 Analysis of the spleen and mucosa microbiota using next generation sequencing (NGS) in the neurodevelopmental rat model of schizophrenia

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The aim of this study was to evaluate the composition of the spleen and gut mucosa microbiota in the rat model of schizophrenia.
Materials were tissue samples taken from rats and divided into two groups: 1. animal model of schizophrenia (SCZ) and 2. control group (CTR) – duodenal tissue (SCZd n = 14, CTRd n = 10), colon tissue (SCZc n = 14, CTRc n = 10), spleen (SCZs n = 13, CTRs n = 10).
Bacterial DNA was isolated from the specimens and 16S libraries were amplified by PCR. Sequencing was carried out using the NGS method (Illumina 16S protocol and MiSeq platform). At the phylum level (L2), the dominant bacteria in all groups were Actinobacteria except SCZc and SCZc (25.64%) vs CTRc (50.61%), pFDR=0.002. At L6 (genus level) the bacterial profiles were varied but only the percentage of bacteria belonging to *Pseudomonas* (1.75% vs 0.03%, pFDR=0.0004) significantly differed in SCZd compared to CTRd group, respectively. The microbiota composition is similar in CTRs and SCZs but the percentage of bacteria belonging to the genera: *Escherichia*, *Shigella* 4.29% vs 0.41% pFDR = 0.0008, *Cutibacterium* 0.46% vs 0.001% pFDR = 0.004 and *Cellulosimicrobium* 63.84% vs 20.77% pFDR = 0.04) were significantly higher in SCZs than in SCZc, respectively.
Differences at L6 in the bacterial profile between the SCZd and the CTRd groups suggest that there may be an association between specific bacterial species and schizophrenia, and it concerns the small intestine rather than the large intestine. The presence of bacterial DNA in spleen samples may indicate the translocation of microorganisms from the intestine to this organ due to permeability of the intestinal wall but the permeability in the SCZ appears to be greater than in CRT for selected genera of bacteria.
This study was supported by National Science Centre (Poland) – project grant no. 2019/03/X/NZ5/00953.

Monitoring the gut microbiome in the course of prostate cancer in mice P048

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Prostate cancer (PCa) is one of the most common cancers among men in the world, therefore attempts are being made to understand the pathomechanism and course of this disease, incl. data on PCa and gastrointestinal microbiome. Here, the factors disturbing the bacterial homeostasis were analysed by chemical markers, i.e. short-chain fatty acids (SCFA) and 3-hydroxy fatty acids, markers of lipopolysaccharide to assess the inflammatory potential of gut microbiota in the course of PCa.

The levels of SCFAs and 3-OHFAs in the stool of mice with induced PCa (TRAMP-C1 or TRAMP-C2 cells) were determined to reveal intestinal microbiota homeostasis disorders related to a disease progression. An analytical approach: HPLC with UV/Vis and GC-MSMS provided the laboratory platform to follow the microbiome changes in the course of PCa.

The most apparent changes were recorded on days 21–40 of the experiment, when the growth of neoplastic tumors was the highest. A decrease in the number of *Pseudomonadales*, *Bacteroides* and *Fusobacteriales* in the stool samples for the prostate tumor model group compared to the control group was observed.

Around days 21–40, the animals developed neoplastic tumors of the prostate at a very vigorous pace, likely leading to an increase of butyric acid and acetic acid in mice with TRAMP-C1 (day 21) and TRAMP-C2 (day 40).

The analysis of SCFA and chemical LPS markers provided a quick, inexpensive tool for microbiome assessment, complementary to DNA sequencing, offering an interesting target for research on microbes-host-cancer interaction.

P049 A high-fiber diet and the gut microbiota in the course of colorectal cancer

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The accurate composition and metabolism of the intestinal microbiome to the greatest extent depend on the diet. An adequate intake of dietary fiber results in a large production of short-chain fatty acids (SCFA), contributing to the anti-inflammatory reaction by participation in maintaining a balance between the suppression of inflammatory mediators (TNFα, IL1β, IL6) and the induction of anti-inflammatory cytokine (IL10).

Here, we have determined the levels of SCFAs in the stool of mice with colorectal cancer and a control group to assess microbiota homeostasis disorders related to a cancer progression, as well as the influence of high-fiber diet (cellulose or potato starch) on the concentration of SCFA in feces and cancer progression.

Analytical methods (HPLC with UV/Vis) and NGS sequencing were applied.

The diet was the main modulator of the gut microbiome: a diet rich in cellulose did not increase the fecal SCFA concentration except of lactic acid. Moreover, a diet rich in potato starch significantly changed the SCFAs profile and increased their fecal concentration.

A diet enriched with cellulose had a positive effect on an intestinal abundance of *Akkermansia muciniphila* in cancer-affected animals. *Lactobacillus* abundance decreased when diet enriched by 20% cellulose was used. Animals consuming a cellulose-rich diet reveal a lower *Firmicutes*: *Bacteroidetes* ratio compared to the group on a standard diet.

A diet with potato starch (type 2 resistant starch) caused an increase of *Bifidobacterium* and *Faecalibaculum* bacteria, and reduced abundance of *Blautia*, *Peptococcus*, *Ruminococcus* UCG-010 and *Anaeroplasma*.

City traffic in Jerusalem Avenue, Warsaw, 1935.

Photo by Henryk Poddębski, The Museum of Warsaw.

IV. T-herapy

Antimicrobial Drugs and Treatment Strategies

P050 Antistaphylococcal enzybiotics – new disinfectants of food production surfaces

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Sustainable and safe food production requires development of novel technologies and innovative solutions at interconnected stages of the food chain: from production and harvest to processing, storage, preparation and consumption. Food safety control faces many challenges which should be immediately addressed, including reduction of development, and spread of antibiotic resistance bacteria in the food chain, and discovery of novel, targeted strategies towards emerging pathogens which have a high impact on human health as well as on the food industry. One of the top five major pathogens responsible for food borne illnesses globally is *Staphylococcus aureus*, causing staphylococcal food poisoning (SFP). Bacterial strains transmitted in the food chain show both, antibiotic-resistance and tolerance to disinfectants. There is a need for environmentally friendly alternatives to control *Staphylococcus* spp. and other bacteria in food which processing environments that enable sustainable production and consumption of food that is safe. Enzybiotics, the enzymes combating pathogenic bacteria, are mainly peptidoglycan hydrolases of phage-origin (endolysins), but can be also recruited among class IIIa bacteriocins, bacteriolysins (e.g., lysostaphin from *Staphylococcus simulans*) and autolysins, like LytM from *Staphylococcus aureus* (Auresine) fused with lysostaphin SH3b domain (AuresinePLUS). Exceptional effectiveness of both enzybiotics in eradication of *Staphylococcus aureus* cells were tested, both as planktonic cells and biofilms. Auresine and AuresinePLUS are considered as safe, stable and universal disinfectants, which are active in a wide range of temperatures, pH and conductivity environments.

Biological activity of gemini quaternary ammonium salts against selected microorganisms

P051

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The widespread use of disinfectants may generate the resistance of microorganisms to these compounds. Therefore, there is a need to design and synthesize new chemicals with bactericidal and fungicidal activity. Gemini quaternary ammonium salts have great potential in this field.
The biological activity of the group of gemini quaternary ammonium salts (QAS) (bromides, with 12 carbon alkyl chains and with different link lengths) against selected microorganisms was tested. All tested compounds had bactericidal and fungicidal activity. Surfactants with 8 and 10 carbon atoms in the linker (2 × $C_{12}L_8$ and 2 × $C_{12}L_{10}$) had the best activity. Moreover, it was found that the tested QAS eradicated the biofilm formed by *Staphylococcus epidermidis*, *Escherichia coli* and *Candida albicans*. Also, these compounds reduced the adhesion of microorganisms to polystyrene, stainless steel, silicone and glass surfaces. In order to assess the safety of the tested surfactants, their cytotoxicity, mutagenicity and haemolytic activity were examined. The obtained results indicate that the tested series of surfactants has a high application potential. The compounds inhibited microbial growth at low concentrations, had strong activity against planktonic and biofilm forms. None of the tested surfactants were mutagenic and cytotoxic.
The research was supported by the National Science Center grant OPUS 16, no. 2018/31/B/NZ9/03878 (Principal investigator E.O.)

P052 The development of conclusive toolbox for analysis of *in vitro* antimicrobial activity of Essential Oils

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The overuse of antibiotics, especially in the form of monotherapy, induced rapid increase of microbial resistance and decreased usefulness of these drugs in the clinical setting. In turn, plant-derived Essential Oils (EO) composed of broad spectrum of antimicrobials, which combined activity and non-specific mechanism of action not only led to microbial death but also did not allow the development of resistance patterns. Nevertheless, due to such variables as plant growth conditions, extraction procedures, differentiated methodologies of antimicrobial testing, improper number of strains analyzed, the results on antimicrobial activity of the same EO against the same microbial species, reported by various research teams, are often of a contradictory nature. Such a phenomenon is one of the main obstacles hindering the introduction of EOs' active compounds into the clinical setting. To overcome this challenge, we experimentally scrutinized the aforementioned variability factors behind it and developed an approach which includes pharmacopeial requirements, *in vitro* biofilm culturing methodology, and impact of intra-species variability. By using this toolbox, the obtained results on EOs antimicrobial testing displayed high repeatability and higher control of experimental conditions. Our results may be considered an important step paving a way for future introduction of EOs' active compounds into the fight against clinical infections.

P053 Phage-derived proteins with anti-microbial activity against *Klebsiella pneumoniae*

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Klebsiella pneumoniae is usually a harmless bacterium, colonizing human mucosal surfaces of the oropharynx and gastrointestinal tract. However, it can turn into a "superbug", impossible to fight with common antibiotic treatment. Infection may be fatal if bacteria infect the lungs (causing pneumonia), urinary tract, brain (meningitidis), heart (peritonitis), blood (bacteremia and sepsis) or chirurgical wounds. Alternative treatments of *K. pneumoniae* infection are currently intensively investigated.

Recently isolated bacteriophage Pra33, specific for *K. pneumoniae*, was sequenced and its genome was examined *in silico* for the presence of genes encoding proteins with bactericidal activity. We were looking for endolysins (peptidoglycan hydrolases), holins (disturbing cellular membrane) and depolymerases, which are often phage structural proteins with enzymatic activity, able to degrade any external polymers (such as polysaccharides). All such proteins may be used as therapeutic antimicrobial agents.

We selected 5 candidate genes, potentially encoding wanted enzymes. ORF19 potentially encodes a phage endolysin with predicted amidase activity, ORF46 probably encodes a holin. ORF39, ORF44 and ORF45 were selected as encoding structural proteins with potential enzymatic activity.
All ORFs were cloned on pET28(+) vector and expression of recombinant his-tagged proteins was induced in different permissive *Escherichia coli* strains (Xpress, BL21DE3, Rosetta, ER2566) in different temperature conditions (20, 30 and 37°C) for 2 hours. Prepared crude extracts were applied to lawns of *K. pneumoniae*, *Pseudomonas aeruginosa* and *Bordetella*. Lysis zones were observed on *Klebsiella* lawns for proteins encoded by ORF39 and ORF45. ORF19 activity will be further investigated in presence of outer membrane permeabilizators.

How the order of administration of ciprofloxacin, caspofungin, and bacteriophages influences *Staphylococcus aureus*/*Candida albicans* dual-species biofilm eradication

P054

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We are living in a post-antibiotic era where the lack of efficient drug therapy affects patients worldwide. Therefore, alternative solutions are investigated, including phage-antibiotic synergy (PAS). However, this approach needs analysis of how bacteriophages and antibiotics cooperate. The effectiveness of PAS therapy depends on many factors, e.g., dosage of antibiotics and phages or time and order of administration.
In this study, we examined how the order of administration of ciprofloxacin (CIP), caspofungin (CASP), and phage cocktail (vB_SauM-A, vB_SauM-D (AD)), influences the efficiency of *Staphylococcus aureus* and *Candida albicans* dual-species biofilm eradication. 24 hours old *S. aureus*/*C. albicans* biofilm was formed on 96-wells plates. Then antibiotics (CIP 8 mg/l (1 × MBEC80); CASP 0.2 mg/l (1 × MBEC80) and phage cocktail (AD) approx. 1×10^7 PFU/ml were added simultaneously or in 6-hour or 24-hour intervals. After curation, qPCR, viability assay, and biofilm biomass assay were performed.
The results show that there is more than one favorable combination, and the optimal treatment is changeable depending on the time of intervals. When the time interval equals 6 hours, the best combination was simultaneous application (AD+CIP+KAS) or the sequence (AD+CIP t = 6h KAS). In the case of 24 hours, the best eradication of both species was obtained using the sequence: CASP t = 24h AD+CIP.
In conclusion, the order of administration of antibiotics and phages is critical for successfully eradicating *S. aureus*/ *C. albicans* biofilm. This aspect should be considered when planning alternative therapies based on the synergistic action of drugs and bacteriophages.
Funding: National Science Centre, Poland [PRELUDIUM 19, Project No. 2020/37/N/NZ9/02947].

P055 Bioactive microfibres and nanofibres as potential dressing materials for bacterial wound cleansing

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Drug-resistant bacteria are a problem in hospitals, threatening the lives of people with severe infections and hampering the wound healing process by prolonging wound inflammation. For this reason, innovative solutions are being sought, including materials for cleansing wounds, i.e. eliminating drug-resistant bacteria from wounds and thus accelerating wound healing. High hopes for the development of innovative materials that could in future form the basis for the development of dressing materials to combat drug-resistant bacteria infecting wounds lie in micro- and nanofibres obtained in an electrostatic field. The main advantage of micro and nanofibres is their developed specific surface area and their ability to be combined with temperature-sensitive substances. For this reason, they are excellent candidates for the development of bioactive fibres. In the present study, microfibres and nanofibres were obtained from combinations of biodegradable polymers such as polylactide, polyethylene oxide and polycaprolactone with materials of natural origin, e.g. bromelain, synthetic origin, e.g. copper nanoparticles; The synthesized materials were analyzed for antibacterial activity according to PN-EN ISO 20645 standard. The obtained fibres in the form of a non-woven fabric were tested with two reference bacterial strains (*Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 11229).
The obtained results allow the selection of materials showing antibacterial activity and promising hopes for the development of dressing materials that could effectively protect against drug-resistant bacteria.

P056 Structural and biochemical characterization of M23 peptidases as promising components of antimicrobial agents

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M23 peptidases represent a class of enzymes able to cleave bacterial cell wall by selective hydrolysis of peptide bonds between amino acids that form the major structural component of the cell wall – peptidoglycan (PG). M23 peptidases belong to a broader group of enzymes, called peptidoglycan hydrolases, which also include amidases, muramidases and glycosidases. M23 peptidases display a very conserved fold with characteristic sequence motifs HxxD and HxH involved in zinc coordination, but their spectrum of lytic action is broad and includes both Gram- positive

and Gram-negative bacteria. M23 peptidases have various functions starting with autolytic role crucial during cell expansion and division to elimination of other bacteria residing in the same ecological niche.
Our research is focused on the biochemical and structural characterization of M23 peptidases in the context of their structure-function relationship and unique antimicrobial potential against drug-resistant pathogens. We modulate and engineer their features to develop perfect antibacterial weapons, so called enzybiotics (therapeutic enzyme-based antimicrobials).
We have performed *in silico* analysis of more than 90,000 protein sequences containing conserved M23 motifs taking into consideration, among others, their modular architecture and surface charge. Based on this analysis, we created a platform for designing enzybiotics composed of a M23 catalytic domain accompanied by various cell wall binding domains. We selected the chimeric enzymes to have desired features in terms of activity, selectivity, safety and stability in a broad range of environmental conditions.

Investigation of the protective effect of monoclonal antibodies against poly-N-acetyl glucosamine (PNAG), a superficial polysaccharide, as an alternative to antibiotic-containing eye drops for the prevention of neonatal eye infections caused by *Neisseria gonorrhoeae* P057

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Neisseria gonorrhoeae can cause eye infections and permanent blindness, especially in newborns. Here, we aimed to develop novel treatments for the prevention of these infections. Strains collected from patients with *N. gonorrhoeae* eye infections were obtained from investigators at the Harvard Medical School, Brigham and Women's Hospital. They were studied for their susceptibility to serum bactericidal (SBA) killing by a monoclonal antibody labeled F598, specific for the conserved microbial surface polysaccharide, poly-*N*-acetyl glucosamine. In the SBA, killing of the strains was evaluated using various concentrations of the antibody, the target bacterial suspension and an antibody-depleted human complement source. Monoclonal antibody (MAb) F429, was used as negative control. Protective efficacy was evaluated in a mouse model of conjunctivitis. Seven to eight week old BALB/c mice were infected with strains and doses of *N. gonorrhoeae* to establish the model of infection. Pathology scores were quantified, using a scale of 0 (no pathology) to 4 (rupture of the ocular surface). After 36–48 hours the eyes were recovered, homogenized and the bacterial levels determined. Studies were then carried out to investigate the effect of MAb F598. For this, MAb F598 was given IP 8 hours after the infection along with applied topically at 8, 24 and 30 hours after the infection. As a result, it was determined that MAb F598 mediated potent SBA activity and use of the antibody in infected mice both decreased the pathology scores and the bacterial levels. These findings indicate a potential strategy for treatment and possibly prevention of gonococcal conjunctivitis.

P058 Superabsorbent crosslinked bacterial cellulose as an antibiofilm dressing material for highly exuding wounds

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Highly absorbent, antibacterial dressings with a sustained release of antimicrobials are considered necessary measures to counteract biofilm-based, chronic wound infections. Currently, the wound dressing market is focused on the use of biopolymers thanks to their biocompatibility, biodegradability, durability and the possibility of targeting their properties by introduction of various types of modifications. One of such biomaterials is bacterial cellulose (BC), synthesized by the non-pathogenic bacteria *Komagataeibacter xylinus*.
This study aimed to develop and evaluate the applicability of BC modified by chemical crosslinking, and impregnated with an antiseptic based on octenidine dihydrochloride (OCT) as the antibiofilm, highly absorbent dressing material.
The obtained materials were characterized in terms of their structure, cytotoxicity, exudate absorption capacity, kinetics of OCT release as well as antibacterial activity against planktonic and biofilm-forming bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*).
The performed analyses revealed that modified BC materials had a sponge-like, multilayer spatial structure with numerous air spaces and cavities, which affected its physicochemical properties. Crosslinked BC was characterized by high exudate absorption capacity, prolonged (desired) OCT release (as compared to the unmodified BC), high antimicrobial activity against planktonic and biofilm cells for up to 7 days and showed no cytotoxicity against fibroblast cell line L929.
The obtained results allowed to conclude that crosslinked BC impregnated with OCT may be a particularly promising dressing material, especially in the treatment of wounds that are highly-exuding and infected with biofilm-forming bacteria.
This research was funded by National Science Center (Grant No. 2017/27/B/NZ6/02103).

Zebrafish as a model for testing new antibacterial drugs P059

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The year 1822 was the year when Louis Pasteur was born. It was also the year when, for the first time, zebrafish (*Danio rerio*), a small fish from the carp family, was classified and described by Francis Buchanan-Hamilton. For 30 years, zebrafish have been gaining increasing popularity, becoming very popular lab animals. Their genetic similarity to humans, the small-sized and transparent body of embryos rapidly developing *ex utero*, easiness of genetic manipulation, and suitability for large drug screens make zebrafish particularly useful for biomedical studies. In the context of microbes, zebrafish have been proven as an excellent model for studies on bacterial, fungal, viral, and protozoan infections, which uncovers insights into host-microbe interactions and is suitable for studying vertebrate microbiome assembly, dynamics, and function. Zebrafish also emerged as a powerful model for toxicology. We use zebrafish to address the stability, activity, selectivity, and cytotoxicity of innovative antimicrobial substances, which eliminate pathogenic bacteria without affecting the natural microflora. We will present results from tests in which two routes of administration (immersion and injection into the bloodstream) in healthy and infected zebrafish were used to check whether enzybiotics developed in SafeFoodCtrl project (NOR/POLNOR/SafeFoodCtrl/0034/2019), and bacteriolytic enzymes and bacteriocins generated within PrevEco project (NOR/POLNOR/PrevEco/0021/2019) can enter field trials. In both projects, we develop protein-based technologies aiming at the elimination of pathogenic antibiotic-resistant bacterial strains that are transmitted in the food chain as well as the prevention and treatment of bacterial infections in animals, and possibly also in humans.

P060 Bacteriophage influence on metabolic state on human fibroblasts in light of phage therapy's safety of skin infections

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Bacteriophages are seen as an alternative to antibiotic therapy to treat nosocomial infections caused by multidrug resistant bacteria, including multidrug resistant *Staphylococcus aureus* (MDRSA). MDRSA strains are known for their tendency to cause burn wound or surgical wound infections. Phage therapy as an experimental therapy of wound infections has been reported as successful and safe. However, in the light of discovered interactions of phages with eukaryotic, it is crucial to carefully examine the influence phage therapy may have on the patient. Therefore, we have decided to analyze the influence of three bacteriophages active against clinical MDRSA strains on metabolic condition of normal human fibroblasts. We have analyzed cell viability via MTT test and measurements of ATP-levels. We have also analyzed membrane integrity of the cells by measurement of LDH levels after incubation with phages. We have observed that bacteriophages did not raise the levels of LDH released from the cells, therefore we assume the membrane was not compromised by the phages. However, we have observed that high concentrations of two phages: vB_SauM-A and vB_SauM-D had a cytostatic effect on human fibroblasts. This effect was not observed for phage vB_SauM-C. Based on our observation we assume that phage influence on human fibroblasts is dependent on phage type and phage titer. Although we did not observe any negative influence of phages on cell metabolism, we propose that phage doses used for therapy should be carefully chosen not only for their effectiveness but safety as well.
Funding: task no. 0003384

P061 The effect of antibiotics on the bacterial cell surface and outer membrane

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One of the most frequently isolated bacterium from hospitals' patients is *Pseudomonas aeruginosa*, which exhibits high intrinsic resistance to many structurally diverse antibiotics. The outer membrane is a structural feature of all Gram-negative bacteria, and it acts as an auxiliary protection barrier inhibiting penetration of the cell by toxic compounds such as antibiotics.
The aim of the research was to study the effect of selected antibiotics with different mechanism of action on the bacterial membrane from *P. aeruginosa* as a model organism. Polymyxin B and ciprofloxacin were used in our experimental work.

Research investigations assumed to obtain data about cell surface hydrophobicity by Congo red binding assay (CRB) and membrane permeability via Crystal Violet assay (CV). The differences in the effects of antibiotics on *P. aeruginosa* outer membrane (OM) were largely defined by their ability to penetrate into the cell. The permeation through the OM of *P. aeruginosa* was higher for polymyxin B than for ciprofloxacin. In addition, the results showed the lower hydrophobicity of the *P. aeruginosa* cell in the case of treatment with ciprofloxacin solution than with polymyxin B. The characteristics of the *P. aeruginosa* OM to treatment with different type of antibiotic solutions should help in the design or further modification of those antibiotics for successful therapeutic strategies against antibiotic-resistant pathogens.

The research was funded in whole by the National Science Center Poland, grant no. 2020/39/B/NZ9/03196

The effect of newly synthesized gemini quaternary ammonium salts with different counterions on viability, adhesion and biofilm of microorganisms

P062

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Quaternary ammonium salts (QAS) have different properties. They are used in medicine (disinfectants, gene or drug carriers), agriculture (fungicides, biocides) and industry (preservatives, detergents). Their widespread use may make microorganisms resistant to these compounds. For this reason, it is necessary to synthesize new QAS chemical structures with bactericidal, fungicidal, anti-adhesive and anti-biofilm properties.

The biological activity of two groups of gemini QAS, bromides and methyl carbonates with different alkyl chain lengths (C12, C16) against Gram-positive and Gram-negative bacteria was investigated. The best inhibitory and bactericidal activity was shown by bromide with 12-carbon alkyl chains (C12) against *Staphylococcus epidermidis* ATCC and clinical strains. Moreover, the tested surfactants had the ability to eradicate the biofilm produced by both *S. epidermidis* strains and *Pseudomonas aeruginosa* type strain. Compounds having 16 carbon atoms in the alkyl chains showed a stronger anti-biofilm effect than C12. The studies also showed that the deposition of gemini QAS on a glass surface reduced the adhesion of the tested strains to such surface. The best anti-adhesive effect was shown by bromide C12.

In order for these compounds to be used in medicine, they were also tested for hemolysis against sheep erythrocytes, cytotoxicity and mutagenicity. It was shown that C12 methyl carbonate did not lead to haemolysis. Moreover, the tested gemini surfactants did not show any cytotoxic or mutagenic activity (in concentrations ¼ MIC).

The research was supported by the National Science Center grant OPUS 16, no. 2018/31/B/NZ9/03878 (Principal investigator E.O.)

P063 Experimental verification of conservation of amino acid sites under purifying selective pressure in *Mycobacterium tuberculosis*

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Amino acid sites under purifying selective pressure are promising binding sites for antibacterial inhibitors. For such sites, mutations should be deleterious and removed by natural selection. Therefore, inhibitors binding to amino acid sites under purifying selection should be less susceptible to random development of resistance. So far, the conservation of amino acid sites under purifying selective pressure has not been thoroughly experimentally tested. We experimentally verified the cellular significance of selected amino acid sites under purifying selective pressure in RpoB of *Mycobacterium tuberculosis*. We used HyPhy software to identify sites under selection in RpoB in three bacterial populations on different phylogenetic levels – *M. tuberculosis*, mycobacteria, and actinobacteria. We used a genetically modified strain of *M. tuberculosis* to introduce selected substitutions at selected sites into the bacterial genome thru site-directed mutagenesis. Briefly, we introduced plasmids carrying variants of *rpoB* into genetically modified *M. tuberculosis* and confirmed the presence or lack of the mutation through antibiotic screening, followed by DNA sequencing. To date, we have tested 16 amino acid sites. We obtained five *M. tuberculosis* mutants with altered amino acid sites, and we could not obtain mutants for 11 sites despite repeated attempts. We conclude that the conservation of sites under purifying selective pressure is not strict, and we plan to further investigate the sites for which we did not identify mutants.
This work is part of the research project financed by the National Science Center of Poland, grant number 2019/34/E/NZ6/00221.

Potential *Mycobacterium tuberculosis* DNA gyrase inhibitors P064

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Every year about 1.5 million people die from tuberculosis and about 10 million are infected with *Mycobacterium tuberculosis*. A significant problem is the growing drug resistance of bacteria. In 2021, there were over 180,000 cases of tuberculosis caused by drug-resistant strains, and only about half were successfully treated. Therefore, there is a need to search for new antituberculosis compounds. One group of currently used antituberculosis drugs are fluoroquinolones whose molecular target is DNA gyrase – a topoisomerase that controls the topology of DNA. The aim of the study was to select and determine the activity of potential *M. tuberculosis* DNA gyrase inhibitors. Using molecular docking, 171 compounds were selected as potential inhibitors of DNA gyrase. In order to determine the minimum inhibitory concentration (MIC) of bacterial growth in the presence of the chosen compounds, the Alamar Blue test was performed. Out of 171 compounds, 3 bactericidal and 4 bacteriostatic compounds against *M. tuberculosis* H37Rv were selected. The mechanism of action of these compounds was then verified. An *in vitro* test of the ability of DNA gyrase to supercool the relaxed plasmid was carried out in the presence of potential inhibitory compounds. The selected compounds at the tested concentrations did not inhibit the activity of DNA gyrase. The bactericidal compounds, despite being indicated during molecular docking as potential DNA gyrase inhibitors, did not show the ability to inhibit the activity of DNA gyrase. More research is needed to determine the mechanism of action of these compounds.

P065 AuresinePLUS – a new enzybiotic with unique features

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Spreading antimicrobial resistance and the lack of new antibiotics calls for the development of new methods of combating pathogenic bacteria. One that is getting more and more attention are enzybiotics, enzymes which with defined specificity and exceptional efficiency eliminate bacterial cells. These peptidoglycan hydrolases cleave bonds within the bacterial cell envelope leading to instant cell lysis.

AuresinePLUS is an example of a new engineered enzybiotic. It is a chimeric enzyme that was created by fusing the catalytic domain of autolysin LytM from *Staphylococcus aureus* with the cell wall binding domain from lysostaphin produced by *Staphylococcus simulans*. This enzyme in a very selective way eliminates millions of staphylococcal cells in minutes leaving the natural microflora untouched. What differs AuresinePLUS from antibiotics it the low prevalence of resistance development. Unlike many other enzybiotics, AuresinePLUS activity is sustained in serum and milk, which opens possibilities for the practical application of this enzybiotic in veterinary practice and medicine. The engineering of a linker joining the two domains improved the stability and thermostability of the enzyme allowing its implementation in various technological processes. AuresinePLUS is a safe alternative for current antimicrobials and as a biodegradable substance does not pose a threat to the environment.

With its unique features AuresinePLUS can be implemented in various products to prevent and treat infectious diseases in humans and animals.

P066 Structural analysis of cyclic AMP peptide in solution and DPC micele by multidimensional NMR spectroscopy

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Antimicrobial peptides (AMPs) are ubiquitous defensive molecules that protect organisms from bacterial infections. They play a fundamental role in the immune system of plants providing rapid responses to pathogen challenges. Although they share similar physical properties, they reveal various biological activities, limited sequence homology, and high variability in 3D structure.

Wild plants, specifically weeds, represent a potential source of novel potent AMPs that demonstrate enhanced resistance to pathogens. For the time being, AMP peptides from flowers are poorly studied, however, the available data suggest unique structural and functional features for that species. In our group, we synthesize the short (15 residues long) fragment of cysteine-rich

AMP, recently isolated from *Taraxacum officiale* Wigg flowers. To reduce conformational freedom and achieve membrane permeability we perform cyclization of the investigated fragment.
We perform structural analysis of the cyclic AMP utilizing multidimensional NMR spectroscopy. Assignments of 1H, 13C, and 15N resonances were done by the jointed analysis of 2D homonuclear and heteronuclear experiments acquired at a natural abundance of 13C and 15N isotopes. The experimental data were acquired on the sample prepared by dissolving 2 mM peptide in 20 mM TRIS-d11 buffer in 90%/10% H_2O/D_2O. To obtain initial data about interactions with cell membrane, NMR data were collected for peptide in DPC-d38 micele. 3D structures in both media were evaluated on base distance constraints yielded from 1H-1H NOESY spectra recorded with a mixing time of 150 ms. As a result, the structural information can be valuable for developing novel therapeutics and crop protection strategies.

Green support in antibiotic therapy – saponins as natural bioenhancers P067

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The increasing drug resistance of pathogenic microorganisms, the adverse reactions caused by synthetic pharmaceuticals and the rising costs of their production have prompted modern researchers to search for natural but effective methods to support antibiotic therapy. Studies on the use of natural surfactants – saponins – have been undertaken to provide greater bioavailability of antibiotics, allowing strong biocidal effects at relatively low doses. Completed studies included toxicity tests of antibiotic mixtures (including aminoglycosides, 5-nitrofuran derivatives and azole compounds) against bacteria of the genus *Pseudomonas* and fungi of the genus *Candida*, for which the addition of saponins isolated, among others, from the fruit of *Sapindus mukorossi* and the roots of *Saponaria officinalis* was found to reduce the biocidal dose of antibiotics. In order to understand the mechanism of the synergistic effect of saponins and antibiotics, changes in the structure and properties of bacterial and fungal cells were investigated using, among others, such tools as electron microscopy and atomic force microscopy. Furthermore, the results obtained were compared with those collected for model systems of phospholipid membranes in the form of liposomes. The results lead to the conclusion that a higher uptake of antibiotics is supported by saponins. However, the mechanism does not involve the physical–chemical modification of the phospholipids membrane only, because the natural surfactants may also enhance active transport along with passive transport as well. Hence, the study brings new perspectives for effective antibiotic therapy.
This study was funded in whole by National Science Centre, Poland (grant No. 2020/39/B/NZ9/03196).

P068 Comparative effectiveness of consecutive alternating administration of antivirals in Coxsackievirus B3 infection in mice

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Human enteroviruses, distributed worldwide, are causative agents of a broad spectrum of diseases with high morbidity, including a series of severe illnesses that affect the CNS, heart, skeletal muscles, and so on. There is no specific treatment available for these infections, and the patients' treatment is mainly supportive. Our team has developed an experimental treatment strategy based on consecutive alternating application (CAA) of enteroviral inhibitors. This work represents the antiviral activity of triple combinations of anti-enteroviral compounds applied via CAA course against Coxsackievirus B3 (Woodruff strain) (CV-B3) infection in newborn mice. Antiviral combination effects were examined by relying on triple CAA combinations consisting of pleconaril (Pl), pocapavir (Po) and vapendavir (V) (Pl/Po/V) or pleconaril (Pl), MDL-860 (M) and oxoglaucine (O) (Pl/M/O) against CV-B3 infection in ICR newborn mice infected s.c. with 20 MLD_{50}. Cumulative mortality (percentage), mean survival time (MST) (days) and weight (in grams) of suckling mice were recorded.
The results of these analyses indicate improved efficacy of Pl/M/O combination administered according to the CAA treatment schedule in CV-B3 infected mice – decreased mortality rate and extended mean survival time (MST). Pl/Po/V applied consecutively were ineffective. In comparison with placebo groups the monotherapeutic course with pleconaril demonstrated some independent antiviral effect. It was found that pocapavir, vapendavir, MDL-860 and oxoglaucine monotherapies were without a protective effect.

P069 *In vitro* evaluation of double combinations against Coxsackievirus B3 and Poliovirus

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The human enteroviruses (EV) comprise one group of the picornavirus family. These EVs cause a diverse array of clinical features, including aseptic meningitis; hand, foot, and mouth disease; neonatal sepsis-like disease; pancreatitis; encephalitis; myocarditis and pericarditis; paralysis and respiratory diseases. The best known members are the polioviruses (PV) and coxsackieviruses. There currently are no EV-specific drugs available for clinical use. One of the reasons is

fast development of drug-resistant mutants. Synergistic combinations of two agents can overcome toxicity and restrict the emergence of resistance to the partners in the combination.
We studied the combined effects in cell culture of inhibitors with different mode of action against cardiotropic Coxsackievirus B3 (Woodruff strain) (CV-B3) and Poliovirus-1 (LSc–2ab strain) (PV-1). Theoretical additive interactions of expected effects for drug–drug interactions were calculated by using MacSynergy II. Interpretation of significance of the observed volumes of synergy or antagonism, depicted in the differential surface plots, was based upon the program guidelines.
The combinations of pocapavir with oxoglaucine, 2-(alpha-hydroxybenzyl)-benzimidazole (HBB) and pleconaril against CV-B3 demonstrated an additive effect. The combination of pocapavir and oxoglaucine indicate synergistic antiviral activity on PV-1 replication in HEp-2 cells. An additive effect was exhibited when pocapavir was combined with 2-(alpha-hydroxybenzyl)-benzimidazole (HBB) and pleconaril against PV-1. Tested combinations had no cytotoxicity effects on uninfected HEp-2 cells.

The effect of castalagin on HSV-1 infection in mice P070

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Herpes simplex viruses (HSVs) are ubiquitous and known since ancient times. They often infect humans, causing a number of diseases from mild uncomplicated mucosal infections to life-threatening conditions. The treatment of infections caused by HSV-1 and HSV-2, embrace a huge number of antiviral drugs. Recently, of special interest as anti-herpetic agents are tannins, which are a group of polyphenols, divided into two groups of condensed and hydrolysed compounds. One of the groups of hydrolysable tannins is the ellagitannins. There is a lot of evidence in the literature that different types of ellagitannins show anti-herpesvirus activity.
In this study, we tested the *in vivo* anti-herpetic effect of castalagin, an ellagitanin compound, extracted from *Quercus robur*, towards HSV-1 infection in newborn mice. The therapy courses with castalagin included groups receiving different daily doses: 20 mg/kg, 10 mg/kg, 7.5 mg/kg or 5 mg/kg of compound.
Acute toxicity determination in mice showed i.p. LD_{50} value of 295 mg/kg. Toxicological picture of intoxication as well prolonged toxicity was done as well. The compound manifested a marked activity at HSV-1 intracerebral infection dose of LD_{90}/0.02 ml when administered in a 7 days course at s.c. (7.5 and 10 mg/kg). Some protection effect was recorded at 20 mg/kg. The dose of 5 mg/kg was ineffective. A reference course of acyclovir demonstrated a marked activity at a daily dose of 20 mg/kg.

P071 The chemical structure of cationic surfactants and their biological activity against microorganisms

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Quaternary ammonium salts (QAS) are one of the compounds widely used in medicine and various industries. They are able to eradicate biofilm and coat surfaces, preventing the adhesion of microorganisms. The study investigated the biological activity of a group of newly synthesized QASs against selected microorganisms (bacteria and fungi).

The chemical structure of the five tested compounds, which are derivatives of benzoic acid, allows them to be divided into three main groups differing in structure – one monomeric compound with a simple structure, one multifunctional with two chains and three gemini differing in the length of the linker.

The study determining the MIC (minimal inhibitory concentration) and MBC / MFC (minimal bactericidal / fungicidal concentration) showed the highest effectiveness of the monomeric compound, especially against Gram-positive bacteria.

The adhesion ability of microorganisms to abiotic surfaces (stainless steel, polystyrene, silicone and glass) coated with the tested QASs was investigated. It was shown that the best anti-adhesive activity of the tested compounds was mainly against *Candida albicans* ATCC10231 cells, where the inhibition of adhesion was > 80%.

The tested QASs were able to eradicate a mature biofilm. The monomeric compound eradicated up to 80% of bacterial and fungal biofilms, being the most effective of the studied group.

The above results give hope for the use of QASs tested as effective disinfectants and anti-adhesive compounds, but their application potential may be limited by their safety for the human body. These studies showed that the compounds are not mutagenic but weakly cytotoxic or haemolytic.

Enzybiotics – efficient food preservatives: eradication of staphylococci from food products

P072

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Global demand for food is expected to grow by 70% in the next 30 years (FAO), which will require a substantial increase of food production. The fulfilment of this demand comes at a huge environmental cost and many of the natural resources already show signs of degradation or are used unsustainably. Even if the global food production system is able to deliver enough food, its quality and bacteriological safety will become an issue. Many of the pathogenic bacterial strains transmitted in the food chain are antibiotic-resistant and the application of current food preservation technologies does not reduce the horizontal transfer of antibiotic resistance genes. Alternative infection prevention and food preservation methods should be considered, such as antimicrobial enzymes, called enzybiotics, which specifically target pathogenic bacteria leaving natural microflora untouched. Enzybiotics are mainly recruited from among peptidoglycan hydrolases, which specifically target peptide bonds in the peptidoglycan structure of bacterial cell walls, causing their instant lysis and death. Two enzybiotics, Auresine and AuresinePLUS, targeting staphylococcal strains, were engineered on the basis of *Staphylococcus aureus* catalytic domain of LytM autolysin (Auresine), additionally fused with lysostaphin SH3b (AuresinePLUS) from *Staphylococcus simulans*. The specificity and efficiency of both enzybiotics were tested against many staphylococcal strains, including food isolates and methicillin resistant strains (MRSA). Their ability to eradicate *Staphylococcus aureus* from contaminated food products, e.g., milk, meat, was tested in various temperatures as well. Enzybiotics, Auresine and AuresinePLUS, are considered safe for humans and the environment, which was confirmed in keratinocytes MTT assay, genotoxicity assay and using *Danio rerio* toxicity test.

P073 The increased activity of antiseptic against pathogenic biofilm in the presence of the rotating magnetic field

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The topical application of antiseptic agents for treatment of biofilm-related infections is considered one of the main pillars of chronic wound management. However, due to intrinsic high adaptability, a biofilm may survive exposure to antiseptic and renew within a relatively short time. Of many possible solutions aimed at counteracting this undesired phenomenon, the application of magnetic fields boosting the efficacy of antimicrobial molecules, seems to be of particularly high potential.

The purpose of this study was to analyze the increased activity of octenidine-based antiseptic against *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms in the presence of rotating magnetic field (RMF), in an *in vitro* model consisting of stacked agar discs, placed at increasing distance from the source of the antiseptic solution. The biofilm-forming cells viability, morphology as well as biofilm matrix structure and composition were analyzed.

The exposure to RMF or antiseptic solution separately did not lead to the destruction of biofilm, contrary to the setting in which these two agents were used together. It was also demonstrated that RMF weakened the walls/membranes of biofilm-forming cells, increased the porosity of biofilm matrix and altered its chemical composition. Consequently, the observed effect of the combined antiseptic and RMF application can be described as of an additive nature.

In the future perspective, RMF may find an application as a therapeutic agent increasing the effectiveness of antiseptics, especially in an environment where physical obstacles may hinder their activity.

This research was funded by National Science Center (Grant No. 2017/27/B/NZ6/02103).

Old Town Market Place, Warsaw, 1945

V. E-ngineering

Microbial Biotechnology – Industrial Microbiology – GMO

P074 Physiological response of *Pseudomonas aeruginosa* to prolonged exposition to hybrid nanomaterials

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The interaction between nanomaterials and microorganisms leads to various physiological effects. The usual direction for such studies is a search for antimicrobial effects. Nevertheless, sublethal concentrations of nanomaterials may, in specific cases, stimulate bacteria to produce secondary metabolites. Even though many physiological effects have been studied and described, the influence of nanomaterials on bacteria during prolonged contact remains unclear. This study aimed to describe the physiological effects caused by prolonged exposure of *Pseudomonas aeruginosa* to carbonized hybrid nanomaterials and their components.

The research material consisted of 10 *Pseudomonas aeruginosa* strains derived from *P. aeruginosa* ATCC 27853. The original strain was passaged 50 times with eight different carbon nanomaterials. The selected physiological features of the group (including biofilm formation, respiration rate, growth dynamics, antagonism against reference bacteria, as well as pyocyanin and rhamnolipids production) were tested. Furthermore, susceptibility to antibiotics and biochemical activity of the bacteria were recorded via the VITEK system. Pulse-field gel electrophoresis (PFGE) was used to analyse restriction patterns among the group.

The prolonged exposition to nanomaterials caused a range of physiological changes, including variability in biofilm production and metabolic activity (pyocyanin and rhamnolipids production) that persisted regardless of further passaging without nanomaterials. Surprisingly, the detected differences were not followed by any changes in expressed biochemical markers and antimicrobial susceptibility. Moreover, no changes in PFGE restriction patterns were found. Further molecular studies could reveal the cause of the observed phenomena.

This research was funded by National Science Center (No. 2018/31/N/NZ1/03064).

Efficiency of bio-hydrogen production as a result of dynamics of metabolic activity and composition of dark fermentation microbial communities: open-flow systems vs static batch experiments

P075

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Dark fermentation (DF), a part of acidogenic step of anaerobic digestion, is a promising method of biohydrogen production. However, it has to be remembered that during acidogenesis many other competitive types of fermentations can occur. The point is to elaborate conditions selecting the microbial communities (MCs) rich in hydrogen-producing bacteria (HPB) and maintaining the hydrogen-yielding metabolic pathways including butyric acid and mixed-acid fermentations and other processes such as conversion of lactate and acetate to butyrate. The former results from cross-feeding of lactate, the nutritional interactions between HPB and lactic acid bacteria (LAB) and, as it was evidenced, boosts hydrogen production in the bioreactors. The aims of our studies were: (i) to recognize the biodiversity of DF-MCs able and unable to convert lactate and acetate to butyrate, and to define the conditions for the transformation in batch tests on different media containing sucrose, lactate and acetate; (ii) to refer these results to the metabolic activity and composition of DF-MCs in well- and poorly-performing bioreactors processing molasses to bio-hydrogen in open-flow systems. Both approaches revealed a community balance between HPB and LAB (~2:1) for stable hydrogen producing systems and efficient conversion of lactate to butyrate. In the open-flow systems the balance stems from long-term selection of the MCs, operating conditions such as bioreactor construction, packing material, hydraulic retention time and substrate concentration. A multiple predominance of LAB over HPB or clostridia over LAB results in a metabolic shift from hydrogen-yielding toward lactic acid fermentation or solventogenic pathways, respectively, and decreased hydrogen production.

P076 Changes in *Pseudomonas aeruginosa* physiology after incubation with carbon nanomaterials

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Carbon nanomaterials are becoming popular because of their broad applicability. Increasing their usage raises the risk of their release into the environment, where these materials interact with living organisms, including bacteria. Consequently, it is necessary to examine the interaction between bacteria and nanomaterials to pinpoint the dynamic changes in their physiology. *Pseudomonas aeruginosa* is an opportunistic pathogen that is abundant in the environment. Due to its adaptability and biotechnological potential, it can provide novel insights into the microbial response to nanomaterials.
The study aimed to investigate the changes in *P. aeruginosa* physiology after co-incubation with carbon nanomaterials.
Six carbon nanomaterials were tested using *P. aeruginosa* ATCC® 27853™ strain. A 24-hour chronic toxicity test followed by respiration measurements was performed. Subsequently, microbial growth dynamics were assessed to check the effect of nanomaterials on bacterial growth rates.
The study demonstrated changes in optical density, fluorescence, ability to form biofilm and bacterial viability as a function of carbon nanomaterial concentration. The conducted studies allowed the screening of the basic physiological characteristics of *P. aeruginosa* in response to nanomaterial-induced stress. Differences in the dynamics of bacterial growth were observed. It was concluded that the carbon nanomaterials used showed toxicity toward *P. aeruginosa*. Interestingly, some concentrations can stimulate the viability of these bacteria. The nanomaterials' concentration can stimulate or inhibit biofilm production depending on their type and concentration.
This work was supported by a grant from the Rector of West Pomeranian University of Technology in Szczecin for PhD students of the Doctoral School, grant number: ZUT/31/2022.

P077 Zinc oxide nanoparticles as boosters of pyocyanin production process

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The beneficial role of the bacterial pigment pyocyanin has been recognized in the last decade. This potent chemical can be potentially used in microbial fuel cells, agriculture, animal husbandry,

therapy, and environmental protection. Stimulating pyocyanin production requires different methods, such as modification of medium and process conditions, the addition of various chemical substances, exposure to physical factors, or genetic engineering methods, depending on the bacterial strain used for the studies. Our work aimed to optimize pyocyanin production by the addition of zinc oxide nanoparticles.

Pseudomonas aeruginosa ATCC®27853™ cultured in King A medium for 48 and 72 hours, without agitation, represented the biological material. Pyocyanin production was optimized using a statistical approach (Design of Experiment) with temperature and ZnO nanoparticles' concentration as input factors. The growth, viability, pyocyanin production, glycerol uptake, and zinc ion concentration of the optimized and control culture were monitored.

The collected data indicated optimal conditions for pyocyanin production at 31.5°C and 4.7 µg/ml of zinc oxide nanoparticles. The growth and viability of the culture incubated with ZnO NPs were higher than in the control. The detected zinc ion concentration in the culture medium was increased in comparison to the control. Apparently, pyocyanin production required the application of ZnO NPs in low concentrations. We assume that the released zinc ions might be utilized by *P. aeruginosa* as microelements in biochemical processes intertwined with pyocyanin production.

The research was funded by National Science Centre in Poland, PRELUDIUM 20 (2021/41/N/ST8/01094) "The influence of the stressors on pyocyanin production by *Pseudomonas aeruginosa*".

The selection of cultivation conditions for oyster mushroom (*Pleurotus ostreatus*) P078

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Cultivated mushrooms, such as *Pleurotus ostreatus*, *Armillaria mellea*, and *Lentinula edodes* are just some of the wide range of varieties and species that can be grown at home all year round. The study investigated the impact of changing environmental conditions and the type of substrates on the growth dynamics of the oyster mushroom – *Pleurotus ostreatus*.

Mushrooms were grown on grains of oats and corn, under three different conditions. Variant 1 – a dark and warm room (temperature 20–24°C, no access to light), variant 2 – a bright and warm room (temperature 20–24°C, illuminated daily), variant 3 – a dark and cold room (temperature 18–19°C, no light). Based on the measurement of the mass of the substrate, the best conditions for further cultivation were selected. The tested fungus grew in the form of white mycelium. Cultivation in the sunlight favored fruiting. However, the mycelium in this case was not as compact as in the case of cultivation in a room with no light. During cultivation, a decrease in the mass of the growing medium was observed, which was caused by the use of nutrients from the substrate. The cultivation variant 1 was found to be the most favorable for the cultivation of fungi.

The research presented in the paper was created as part of a specific subsidy entitled: "Technologies and materials and construction solutions from renewable raw materials for modular construction", No. 1/Ł-PIT/CŁ/2022.

P079 Influence of various nanomaterials on Merlot grape must fermentation and yeasts viability

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The role of sulphur dioxide (SO_2) in wine production is multitasking, on the one hand it protects against oxidation and inhibits the development of microorganisms, but on the other hand it may have adverse effect on human health. Therefore, one of the most current research trends in winemaking industry is to eliminate or to significantly decrease an addition of SO_2 in line with unaltered wine properties and quality. The preceding research papers describe plenty potential solutions such as chemical or physical methods to provide as an alternative for SO_2.
One of the latest approaches include using nanotechnology in stabilisation of wine. Our experiment revealed influence of three various nanomaterials (SiO_2, TiO_2 and Fe_3O_4) on Merlot red grape must fermentation and viability of *Saccharomyces cerevisiae* yeasts population. The results showed similar changes in process of converting sugar into alcohol during three weeks period. Afterwards significant differences in sugar (g/L) and thereby in total dry extract (g/L) were observed as a consequence of nanomaterials zeta potentials (ZP). Among tested materials, TiO_2 had positive charge of 36 mV, while SiO_2 had negative charge of −51 mV. Fe_3O_4 showed zeta potential close to point of zero charge (−1.1 mV). In the light of tested parameters, the lower the zeta potential, the lower the sugar and total dry extract contents. Short cytotoxicity tests performed after 30 minutes, 3 and 24 hours showed little impact on yeasts viability, revealing slightly inhibiting or activating effect.
This work was supported by the National Science Centre, Poland, within the PRELUDIUM Program, grant number 2017/25/N/ST5/01222

Synthesis of bacterial cellulose employing enzymatically degraded oligo- and polysaccharides

P080

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Bacterial cellulose (BC) is a versatile biomaterial that, thanks to its unique properties and ability to be modified, has the potential for applications in various industries. However, despite the many advantages of this biopolymer, the cost of its production is still a limiting factor for wider use.
The aim of the study was to check the usefulness of sucrose, lactose, and starch as alternative carbon sources in the culture medium supplemented with microbial glycohydrolases such as β-D-fructofuranosidase, β-galactosidase, and glucoamylase for enhancing of BC synthesis process efficiency.
The obtained results showed high potential of the enzyme-assisted fermentation process that was exceedingly efficient with a yield and synthesis efficiency of BC, higher or comparable to standard Hestrin-Schramm medium with glucose as a carbon source. The fermentation conditions did not significantly affect the stability and catalytic properties of the enzymes used, allowing for effective degradation of analyzed oligo- and polysaccharides to accessible carbon sources for *Komagataeibacter xylinus* cells. The effective substrate's conversion by the used biocatalyst prevented their incorporation into biopolymer structure and also did not affect the material properties of BC.
The application of specific enzymes for the conversion of carbon source inaccessible in raw form to the culture medium of *K. xylinus* opens a simple way for the use of various oligo- and polysaccharides in the BC production process acquired from many kinds of biomass sources such as *inter alia* food processing waste.

P081 Real-time microbial contamination detection system

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Bacterial culture methods, e.g. the plate counting method (PCM), are a gold standard in the assessment of microbial contamination in multitude of industries. They are, however, slow, labor intensive, and prone to manual errors. Dielectrophoresis (DEP) has shown great promise for particle separation for decades; however, it has not yet been widely applied in routine laboratory settings. This poster shows an overview of a new microbial contamination method and system called Fluid-Screen (FS) which is fast, efficient, reliable, and repeatable. Method verification experiments demonstrated that the FS system limit of detection (LOD) is at 1 CFU/ml and can be applicable to a broad microorganism spectrum.
Fluid-Screen system test takes 1–6 hours rather than 5 to 14 days as compared to the established PCM.

The impact of orphan histidine kinase CpkM on cpk gene cluster transcription and coelimycin production in *Streptomyces coelicolor* A3(2) P082

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Streptomyces, soil-dwelling, Gram-positive bacteria, are prolific producers of specialized metabolites. One of the metabolites produced by the model organism *Streptomyces coelicolor* A3(2) is the yellow polyketide – coelimycin (yCPK). Genes necessary for its biosynthesis form a silent biosynthetic gene cluster (BGC). Its expression occurs only when certain environmental conditions are met and is dependent on quorum sensing. The initiation of BGCs expression in *S. coelicolor* A3(2) also depends on extracellular signals transmitted via two-component systems (TCSs). TCSs couple the environmental stimulus of a sensor kinase (SK) to an adaptive response through phosphorylation of a cognate response regulator (RR), which exerts a regulatory response. Since CpkM does not have assigned response regulator, it belongs to the group of orphan histidine kinases.

In order to establish the impact of CpkM on *cpk* genes transcription activity, an *in vivo* luciferase-based reporter assay was applied. The chosen promoter regions from *cpk* cluster were cloned upstream of *lux* genes and their activity was measured in a real-time mode in the wild type and deletion mutant strains. Additionally, detailed phenotyping on several media has been done.

Altered promoter activity profiles, as well as earlier yCPK production by the *cpkM* deletion mutant, strongly support hypothesis that CpkM is significant for *cpk* cluster expression regulation. Collected data suggest that CpkM may influence *cpk* gene transcription by modulating the action of *cpk* cluster-encoded activators CpkO or CpkN, which belong to SARPs (*Streptomyces* Antibiotic Regulatory Proteins).

Constitution Square, Warsaw, 1950s.

VI. U-biquity

Environmental Microbiology & Microbial Evolution: Microbial Populations and Their Interactions in the Environment – Extremophiles – Pollution Microbiology – Microbial Diversity & Evolution

PICOTA: an extendible pipeline for identification of composite transposons from assembly graphs P083

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One of the mobile genetic elements with a huge impact on bacterial evolution are composite transposons. Composite transposons have flanking insertion sequences responsible for transposition and can mobilize a region of DNA that codes various proteins. This DNA region mostly contains valuable genes related to antibiotic resistance, xenobiotic degradation, virulence, and metabolic functions. Therefore, it is crucial to conduct more comprehensive studies about composite transposons to understand the spread of genes that they carry among bacteria. Massive sequencing data deposited in public repositories are a resource for understanding the role of composite transposons on spreading genes but there is no comprehensive tool to utilize sequencing data for the correct identification of composite transposons. Existing methods are just capable of processing a maximum of 3% of the available sequencing data because of their methodological limitations. Also, those tools cannot identify novel composite transposons. Here we present *PICOTA* algorithm and a software tool to identify these elements from assembly graphs. *PICOTA* can work with almost all raw data of meta and whole genome sequencing projects. It is not only capable of identifying existing ones, but it can also deduce those that are likely to be novel. One of the advantages of *PICOTA* is that it does not need training data. We used *PICOTA* on raw metagenome sequence data of four disinfectant enriched microbial community and two disinfectant degrading strains. We found that genes related to antibiotic resistance and disinfectant degradation were in those samples associated with composite transposons, especially Tn3 family ones.

P084 Metabolic pathways of acetate, butyrate, lactate, and propionate in methane-yielding microbial communities – metagenomics and isotopic approaches

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During the acetogenic step of anaerobic digestion, the products of acidogenesis are oxidized to substrates for methanogenesis: hydrogen, carbon dioxide, and acetate. Acetogens and methanogens form closely related syntrophic systems. The aim of our studies was to determine the influence of the universal products of acidogenesis, respectively, lactate, butyrate, propionate, and acetate, on the metabolic pathways of methane formation and to find a core microbiome and substrate-specific species in a mixed biogas-producing system. A new experimental set-up for studying acetogenesis and methanogenesis was elaborated: methane-yielding microbial communities processing artificial media having one dominant aforementioned acidic component in Up-flow Anaerobic Sludge Blanket bioreactors in a long-term system. Surprisingly, the substrates moderately modified the final methane production and substrate utilization, whereas strongly affected the methanogenic pathways. Acetate and lactate favored the acetotrophic pathway, while butyrate and propionate hydrogenotrophic pathway of methane formation was proved by analyses of the stable carbon isotope composition of the biogas and the substrates. Genome-centric metagenomic analysis recovered 31 archaeal and 203 bacterial Metagenome Assembled Genomes (MAGs), mostly unknown and uncultivable. The core microbiome is represented by five MAGs present in high relative abundance (two methanogens: *Methanothrix soehngenii* and *Methanoculleus* sp., three bacterial MAGs classified only at high taxonomic level) and 108 others with a low relative abundance. Substrate-specific species were mostly unknown and not predominant in the microbial communities, thus we hypothesize the substrate may first change the metabolic activity of the bacteria/methanogens, rather than the composition of the microbial community.

Metabolic potential of microorganisms inhabiting differently cultivated fluvisols P085

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Fluvisols are soils developed from fluvial sediments characterized by a great diversity of morphology and properties. They are commonly regarded as fertile soils and are therefore largely used in agriculture.

The aim of this study was to identify the metabolic potential of microorganisms from fluvisols from the Vistula River valley (Lubelskie voivodeship). The study material consisted of three different fluvisols (light – F1; medium – F2; heavy – F3) sampled in two variants: agriculturally used (blackcurrant, *Ribes nigrum* L., cultivation; – A), and not cultivated (meadow; – M).

The diversity of soil microbial metabolic profiles was assessed using the EcoPlate™ of the Biolog® system. Carbon and nitrogen contents of microbial biomass were also determined using the fumigation-extraction method (PN-EN ISO 14240-2, 2011).

The results indicate that the metabolic activity of microorganisms varies both between soils and soil management practices. In light soils, microorganisms from the soil under blackcurrant cultivation showed higher activity (AWCD index), but in medium and heavy soils, microorganism activity was higher in meadow soils. Putrescine and D-malic acid were the most intensively degraded compounds, while β-methyl-D-glucoside was the least. The differences obtained indicate that human activities related to cultivation affect the metabolic potential of the soil microbiome.

P086 The occurrence of *Prototheca* microalgae in soils in Poland

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Prototheca spp. are unicellular, achlorophyllous microalgae widely distributed in nature. The algae have been recovered from plants, water, and other sources usually with high organic matter and moisture content. The aim of the study was to assess the prevalence of *Prototheca* algae in different soil environments in Poland.

A total of 235 soil samples from 11 voivodships in Poland were collected. Each sample was preincubated in liquid *Prototheca* isolation medium (PIM) for 48 h at 30°C, and then plated on PIM agar, and incubated under the same conditions. The isolated strains were subjected to partial *CYTB*-based PCR-RFLP profiling. In parallel, every soil sample was examined for moisture content, electrical conductivity, and pH. Concentrations of total organic carbon, potassium, magnesium, phosphorus, and nitrogen were also determined.

Of the samples collected, only 9 (3.8%) yielded *Prototheca* growth with species isolation rates of 33%, 25%, 16.7% for *P. bovis*, *P. pringsheimii*, and *P. ciferrii*, respectively, and 8.3% for *P. cerasi*, *P cookei*, and *P. wickerhamii*. Interestingly, 3 out of 9 culture-positive samples contained two different *Prototheca* species.

This is the first study to explore the occurrence of *Prototheca* spp. in Polish soils assisted by physico-chemical analysis. The unexpectedly low isolation rate of *Prototheca* spp. from soils contradicts the repeatedly formulated hypothesis that the algae are ubiquitous inhabitants of soil environments. The recovery of *Prototheca*e could not be correlated to any soil characteristics.

P087 Characterisation of lactococcal and leuconostoc strains in terms of potential probiotic properties

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Despite the fact that lactic acid bacteria (LAB) represent a minor proportion of the bacterial community in the gastrointestinal tract, their role within human intestinal microbiota due to interactions of some LAB with the human intestine and their associated health benefits cannot be underestimated. Certain LAB strains with documented health-promoting properties may be applied in the food industry as probiotic cultures. Apart from lactobacilli, there are many other nonpathogenic LAB strains with a reputed “Generally Recognized as Safe” or

a “Qualified Presumption of Safety” status which can be considered for selection of novel probiotic strains. Indeed, there is growing evidence that selected strains of *Lactococcus* and *Leuconostoc* genera, which play an important role in several industrial and food fermentation processes, present probiotic activities in mice models of various human diseases.
In this work, we screened over one hundred unique lactococcal and leuconostoc strains from the Institute of Biochemistry and Biophysics, Polish Academy of Sciences (IBB PAS) Central Collection, isolated from various food products such as different types of raw milk, fermented milk products, kefir grains, plants, fermented plant food and sourdoughs. These strains were characterized in terms of their ability to survive in conditions mimicking the gastrointestinal tract: resistance of bacterial strains to acid and bile salts, adherence to mucus, and regarding other selected probiotic traits like acidification ability, production of exopolysaccharides and vitamins. The results indicated that both groups of bacteria: lactococci and leuconostocs are a good source of potentially probiotic strains with various biological activities.
This research was funded by The National Science Centre (NCN) [2021/41/B/NZ9/02236].

Unveiling the microbiomes in plastisphere derived from microplastics at municipal wastewater treatment settings

P088

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Activated sludge units at municipal wastewater treatment plants (WWTPs) are “hotspots” converging microplastics and anthropogenic pollutants, such as antibiotics. Our study revealed both polyethylene (PE) and polystyrene (PS) microplastics can acclimate biofilms enriched with antibiotic resistance genes (ARGs) and the associated mobile genetic elements (MGEs) in comparison with fine sands as control particles. The combination of 16S rRNA amplicon sequencing and differential ranking analysis revealed that microplastics selectively promoted antibiotic-resistant and pathogenic taxa (e.g., *Raoultella ornithinolytica* and *Stenotrophomonas maltophilia*) with enrichment indices ranging from 1.6 to 3.3. Furthermore, heterotrophic *Novosphingobium* and filamentous *Flectobacillus* accounted for 14.6% and 3.3% on average in the plastisphere. Dominance of these bacterial species may contribute to initial biofilm formation that facilitates subsequent colonization and proliferation of ARB and pathogens, thus amplifying their risks in the receiving environments and beyond. Furthermore, Nanopore MinION sequencing and metagenomic analysis showed a large diversity of genes putatively encoding bacterial laccases in the assembled genomes of *Novosphingobium* species. These laccases may enable the biodeterioration of plastics and release carbons from microplastics to feed the biofilm. Collectively, microplastics can serve as hubs of antibiotic-resistant bacteria (ARB) and pathogens, representing a pressing concern to aquatic biota and human health. They may also acclimate laccase-secreting microorganisms, promoting the decomposition of these persistent waste.

P089 Uncovering and engineering catalytically versatile di-iron monooxygenases for water purification and bioenergy production

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The importance of clean water for public health and green energy for sustainable development cannot be overstated. Water and energy crises are the top two issues of humanity with increasing concerns as the results of the growth of global population, deterioration of natural resources, and change of our climate. In the spirit of addressing these crises head-on, we aim to advance our fundamental knowledge on versatile biocatalysts and develop innovative techniques to mitigate and address grand challenges related to water and energy. Particularly, we discovered, characterized, and engineered novel soluble di-iron monooxygenases (SDIMOs) for water purification and bioenergy production. SDIMOs represent an important family of bacterial enzymes that are capable of oxidizing hundreds of naturally occurring and anthropogenic compounds, including short-chain alkanes (e.g., methane and propane) and aromatic and cyclic hydrocarbons (e.g., toluene and 1,4-dioxane). SDIMOs also exhibit high versatility in substrates that they can react with. Our recent studies have advanced our knowledge on group-6 propane monooxygenases and group-2 toluene monooxygenases. Combining molecular characterization with transformant clones and bioinformatics-based evolutionary analysis, these SDIMOs were identified with significant values to tackle the commingled contamination of 1,4-dioxane and chlorinated solvents frequently found at impacted sites. Through collaboration with biochemists and computation chemists, we employ directed enzyme evolution and *in silica* machine learning to improve the performance of SDIMOs under environment-relevant conditions and extend their ability to produce primary and secondary alcohols from natural gas, rendering a more efficient and greener use of the world supply of energy.

P090 Assessing the probability of disease caused by spring water consumption contaminated with *Salmonella* spp. and enteric protozoa

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Groundwater used as a drinking water source can be contaminated by many pathogens, causing severe diseases. Even if waterborne outbreaks have various etiological agents, the management of public groundwater systems often considers just a few reference microorganisms. Thus, it makes some viruses or enteric protozoa, frequently present in surface waters, remain largely undetected in groundwater. This study assesses the human health risk associated with enteric bacteria and protozoa contamination of spring water in nine areas in Romania. Water samples

collected in September 2022 were analysed for *Escherichia coli* and *Salmonella* spp. contamination. Based on the established ratio between *Escherichia coli* and enteric protozoa, estimated *Cryptosporidium parvum* and *Giardia lamblia* were used in a Quantitative Microbial Risk Assessment (QMRA) framework. Beta-Poisson and Exponential models calculated the consumers' probabilities of infection and illness. Results have shown that the estimated probability of infection from *Salmonella* spp. and enteric protozoa ranged between 3.2×10^{-1} and 1.0×10^{0}. These values were much higher than the WHO reference benchmark for five out of nine springs. The estimated probability of illness due to the *Salmonella* spp. infection was high, with maximum values of 2.0×10^{-1}, while for *Cryptosporidium parvum* and *Giardia lamblia*, the probability ranged between 2.0×10^{-2} and 2.3×10^{-1}. The results indicated that *Giardia lamblia* and *Salmonella* spp. had high probability of causing illness, even if the daily ingested doses of these pathogens were low. This emphasizes the presence of these pathogens in groundwater supplies and the drastic effects of consuming water from these groundwater sources on human health.

Evaluation of microbiological safety of the natural insecticide – azadirachtin P091

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Growing awareness of the consequences of intensive and indiscriminate use of synthetic pesticides is increasingly prompting the search for natural equivalents. These include insecticides of natural origin, such as azadirachtin. Although they are considered relatively quickly biodegradable and of low toxicity to humans and animals, their impact on the microflora of soils and waters remains an open question. Azadirachtin (AZN) is one of the main bioactive triterpenoid compounds that can be obtained from the plant (*Azadirachta indica*). It possesses strong insecticidal and antibacterial activity. This raises the question about AZN's impact on environmental microorganisms. Our study on the bacterial activity of *Pseudomonas aeruginosa* NFT3, and *Achromobacter* sp. KW1 shows a decrease in the metabolic activity of cells followed by an increase in azadirachtin concentration. At a concentration of 0.2 µg/ml, which corresponds to the concentration of the working solution recommended for spraying against insects, a decrease in the activity of cells of the strain *Achromobacter* sp. KW1 by 43% and of the strain *Pseudomonas aeruginosa* NFT3 by over 60% was observed. These changes are very clear and prove the significant toxic effect of azadirachtin on these exemplary bacterial strains. Further studies will allow us to indicate the safest natural insecticides for the environment, which can be used more widely in the future without worrying about the well-being of bacterial microflora, which is very valuable for the entire ecosystem.

This work has been funded by the National Science Center, Poland (grant no. 2021/43/D/NZ9/01201)

ONZ Roundabout, Warsaw, 1960s.

VII. R-esistance

Microbial Resistance to Drugs and Other Stress Factors: Mechanisms of Resistance and Strategies for Control and Management

Functional characterization of TetR-like transcriptional regulator PA3973 from *Pseudomonas aeruginosa* P092

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Pseudomonas aeruginosa, a human opportunistic pathogen, is a common cause of nosocomial infections. Its ability to survive under different conditions relies on a complex regulatory network engaging transcriptional regulators controlling metabolic pathways and capabilities to efficiently use the available resources. *P. aeruginosa* PA3973 encodes an uncharacterized TetR family transcriptional regulator. We applied phenotype analyses, as well as transcriptome profiling (RNA-seq), and genome-wide identification of binding sites using ChIP-seq to unravel the biological role of PA3973.

Transcriptional profiling of *P. aeruginosa* PAO1161 overexpressing PA3973 showed changes in the mRNA level of 648 genes. Concomitantly, ChIP-seq analysis identified more than 300 PA3973 binding sites in the *P. aeruginosa* genome. The 13 bp sequence was identified as the preferential binding site of PA3973.

The PA3973 regulon encompasses genes involved in stress response, including the putative *PA3973-PA3970* operon. *In vitro* analysis confirmed PA3973 interactions with *PA3973*p. Increased expression of *PA3972* and *PA3971* genes in Δ*PA3973* mutant in comparison with WT strain was observed. The ability to generate the Δ*PA3973* as well as Δ*PA3972* PAO1161 strains indicates that the genes are not essential for the growth of *P. aeruginosa*, nevertheless mutants demonstrated impaired growth in the presence of the stress-inducing agents hydroxylamine and hydroxyurea, suggesting the role of PA3973 and PA3972 in pathogen survival.

Overall our results showed that PA3973 has multiple binding sites in the *P. aeruginosa* genome and influences the expression of genes from different functional categories. It acts as a repressor of *PA3972-PA3971* genes, encoding proteins putatively engaged in stress response and virulence.

P093 Plasmid-mediated resistance of *Neisseria gonorrhoeae* strains isolated in 2014–2021

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Antimicrobial resistance (AMR) of *Neisseria gonorrhoeae* has a multifactorial basis. Gonococcal sensitivity profiles and the incidence of resistance determinants vary by geographic region. A structurally diverse group of β-lactamase plasmids, encoding *blaTEM* penicillinases, determines resistance to penicillin. The conjugative plasmids may carry *tetM* encoding tetracycline resistance American- or Dutch-type.

The aim of the study was to determine the plasmid-mediated resistance of *N. gonorrhoeae* clinical strains by phenotypic and molecular methods.

116 strains of *N. gonorrhoeae* from patients with uncomplicated gonorrhea infections isolated in the CMR&A laboratory in 2014–2021 were analyzed. Based on phenotypic methods, AMR testing was conducted using gradient strips (Liofilchem) on MH Chocolate Agar (Liofilchem) according to the manufacturer's instructions and EUCAST 2022 recommendation. Penicillinase-producing strains of *N. gonorrhoeae* (PPNG) were detected by the cefinase assay, while strains with high tetracycline resistance (HLTR) were identified by tetracycline MIC values. The *blaTEM* and *tetM* plasmids, determining the gonococcal resistance to penicillin and tetracycline, were analyzed by multiplex PCR and electrophoresis distinguishing the expected sizes of the produced amplicons.

Plasmid-mediated resistance was detected by phenotypic methods in 27 strains of *N. gonorrhoeae* (23.3%). Among 116 strains, 9 PPNG were identified (7.7%). All of them presented the African-type β-lactamase plasmid. In the group of 25 HLTR strains (21.5%), 13 possessed the Dutch-type *tetM* on conjugative plasmid (11.2%) and 12 the American type (10.3%).

In almost 1/4 of the clinical strains of *N. gonorrhoeae*, plasmid-mediated resistance was detected by both phenotypic and molecular methods.

Restoration of aztreonam *in vitro* susceptibility in metallo-β-lactamase-producing *Enterobacterales* with novel β-lactamase inhibitors

P094

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Recently, we have seen an increase in the global prevalence of bacteria resistant to more and more antimicrobials, including the ones considered the last resort in the treatment of severe infections. One of the growing problems are carbapenem-resistant Gram-negative rods. Extensively drug-resistant (XDR) metallo-β-lactamase (MBL)-producing *Enterobacterales* are considered an essential clinical threat due to the limited therapeutic options narrowed down to the choice between aztreonam and colistin. Unfortunately, bacterial strains can be resistant even to these. We compared the ability of avibactam, relebactam and vaborbactam to restore aztreonam activity in MBL-producing aztreonam-resistant *Enterobacterales* isolates. The collection of 29 MBL-producing aztreonam-resistant *Enterobacterales* from hospitals in Lodz, central Poland, were investigated during the period January-May 2022. Minimum inhibitory concentration (MIC) values for conventional antibiotics were determined using the gradient strip method. MIC values for aztreonam in combination with novel β-lactamase inhibitors (avibactam, relebactam, and vaborbactam) were determined using the gradient strip superposition method. The susceptibility interpretations were determined following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. The most effective combination was aztreonam/avibactam, which restored aztreonam activity in more than ¾ strains, while combinations with relebactam and vaborbactam did so in more than half and almost ⅔ strains, respectively. However, we identified strains that regained aztreonam susceptibility only in combination with inhibitors other than avibactam. The great *in vitro* performance of aztreonam/avibactam combination increases the anticipation for the approval of this antibiotic at the final straight of clinical trials. Nevertheless, other combinations should be considered so that clinicians have the full opportunity to design an appropriate targeted therapy for XDR infections.

P095 Comparison of cefotaxime and ceftazidime as a cephalosporin resistance indicator using MALDI-TOF MS technology

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Antimicrobial resistance (AMR), is currently one of the most common healthcare problems worldwide. MALDI-TOF MS technology has been recently explored as a rapid AMR detection tool. In this study, we investigate the utility of cefotaxime and ceftazidime as cephalosporin resistance indicator.

The study was performed on 62 *Escherichia coli* strains. Based on the MIC (Minimal Inhibitory Concentration) method, 32 strains resistant and 30 susceptible to cefotaxime and ceftazidime were selected. A 1 µL inoculation loop of bacteria (fresh overnight culture) was suspended in 20 µL of the antibiotic solution (0.5 mg/ml) and incubated at 37°C for 3 hours. Subsequently, the tubes were centrifuged and 1 µL of the supernatant was spotted on the MALDI target plate. Peaks were manually selected using flexAnalysis 3.4 software.

32 *E. coli* strains were correctly confirmed as resistant to cefotaxime (414 Da peak, hydrolyzed form). In the case of susceptible strains, 28 out of 30 were correctly assigned (456 Da peak, antibiotic basic form). Overall, 93.3% specificity and 100% sensitivity were obtained. For ceftazidime 24 out of 32 strains were correctly assigned as resistant (442 Da peak, hydrolyzed form) and 27 out of 30 as susceptible (468 Da peak, antibiotic basic form); 90% specificity and 75% sensitivity were reached. The results of the present study showed 96.8% and 82.3% accuracy for cefotaxime and ceftazidime, respectively.

Antimicrobial resistance results obtained for cefotaxime have a higher specificity, sensitivity, and accuracy rate. There is a need for further method optimization to avoid false positive and false negative results.

The assessment of antibiotic resistance of *Enterococcus* spp. isolated from a piggery

P096

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Enterococcus spp. are opportunistic human pathogens responsible for urinary tract infections, skin infections, endocarditis, and bacteremia. Animals and contact with them are an important source of *Enterococcus* spp. infections in farmers, veterinarians, and slaughterhouse workers. A serious threat is the increased resistance of *Enterococcus* spp. to antibiotics.
The aim of the study was to assess the frequency of *E. faecalis* (EFA) and *E. faecium* (EFM) strains in the pig farm environment, to assess their drug susceptibility and to determine the phenotype and genotype of vancomycin resistance. The tested strains were isolated from the surface and from faeces collected in piggery. The genetic similarity of the tested strains was determined by Random Amplification of Polymorphic DNA (RAPD). The antibiotic susceptibility of *Enterococcus* spp. strains was determined using the disk diffusion method. The minimum inhibitory concentration values for vancomycin, teicoplanin, gentamicin, streptomycin and kanamycin were determined. Glycopeptide resistance genotypes was performed by multiplex PCR. From 475 samples taken, 160 (33.7%) isolates were obtained. Among them, 110 genetically different strains were found, 82 (74.5%) EFA and 28 (25.5%) EFM. The largest number of EFA strains (7; 8.5%) showed resistance to imipenem, while the largest number of EFM strains (5; 17.9%) were resistant to ampicillin. Out of the tested strains, 6 (7.3%) EFA and 4 (14.3%) EFM were resistant to vancomycin. Multiplex PCR analysis demonstrated the presence of 4 EFA strains belonging to the VanB genotype, 1 to VanA and 1 to VanD. Among the tested EFS strains, two belonged to VanA and two belonged to VanB.

P097 The influence of increasing antibiotic resistance of Gram-negative rods on the increase of tolerance of the antiseptic agents

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Multidrug-resistant (MDR) strains are becoming an important problem considering the shortage of available and effective antibiotic therapies. Antiseptics are locally applied as antimicrobial agents with a broad spectrum of activity against bacteria.

The main goal of this study was to determine whether the intensified use of antibiotics and antiseptics is correlated to the cross-resistance.

We conducted studies on MDR *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella pneumoniae* clinical strains isolated from patients. We performed the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) test for octenidine (OCT) and povidone-iodine (PVP-I) for reference and resistant strains from each species to different groups of antibiotics.

The studies showed different levels between MDR strains of MIC and MBC for the tested compounds. The highest level of MIC on OCT diluted 256× was shown by some strains of *A. baumannii* and *P. aeruginosa*. In the remaining groups of the studied strains, the highest MIC results for OCT were 512×. These are doses with a concentration below those recommended. Many tested strains show 2–3 and even 4 orders of higher concentrations in the geometric series. For PVP-I, the highest level of MIC (8× diluted) is shown by *P. aeruginosa* strains. *E. coli* and *K. pneumoniae* strains have MICs of 16× diluted PVP-I. In some cases, the recommended effective dose is lower. The values of MBC concentrations were always higher, even reaching 8× starting dilution for *P. aeruginosa*.

These results demonstrate the possibility of bacteria to adapt to selected antiseptics and the possibility of development of resistance mechanisms of the antiseptics.

The effect of rotating magnetic field on susceptibility of methicillin resistant *Staphylococcus aureus* strains exposed to activity of antibiotics

P098

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The study aimed to analyze the impact of the combined use of rotating magnetic field (RMF) with various classes of antibiotics (β-lactams, glycopeptides, macrolides, lincosamides, aminoglycosides, tetracyclines and fluoroquinolones) against *S. aureus* strains. Our hypothesis was that RMF could have an impact on the overall activity of the antibiotics resulting in higher rate of eradication of methicillin-resistant *Staphylococcus aureus* (MRSA) *in vitro*.
The results indicated that the application of RMF combined with antibiotics interfering with cell walls (particularly with β-lactams) translates to increased lethality in microorganisms manifested in the form of the expanded size of staphylococcal growth inhibition zones or in lower minimum inhibitory concentration (MIC) values, compared to the control settings which were unexposed to RMF. Apart from β-lactams, reduced MIC values were also found for erythromycin, clindamycin and tetracycline, ciprofloxacin, gentamicin and teicoplanin. Fluorescence microscopy indicated a drop in the number of cells with intact cell wall after exposure to RMF. These findings were additionally supported by the use of scanning electron microscope and transmission electron microscopy, which revealed morphological alterations of RMF-exposed cells, manifested by a change of shape, drop in cell wall density and cytoplasm condensation.
Taking into account the high clinical need for new therapeutic options effective against MRSA, the data presented in this study represent a high developmental potential and may be considered an important step in the application of magnetic fields to fight infections caused by these microorganisms.
This research was funded by National Science Center (Grant No. 2017/27/B/NZ6/02103).

P099 Identification and analysis of the genetic carriers of antibiotic resistance genes of *Enterobacter* spp. clinical isolates

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Enterobacter is a genus that includes highly virulent pathogenic bacteria, associated with a vast range of diseases, including urinary tract infections, respiratory infections or endocarditis. The prevalence of antibiotic resistance genes (ARGs) among these bacteria is of high concern and poses a serious public health threat. Mobile genetic elements (MGEs), such as plasmids and transposable elements, play a major role in the maintenance and horizontal transmission of ARGs among bacteria. For this reason we investigated the presence of such elements in the genomes of six clinical isolates of *Enterobacter* spp. These were multi-resistant strains, most of them producing broad-spectrum beta-lactamases (ESBL$^+$), isolated from patients hospitalised at the Clinical Hospital of the Medical University of Warsaw (Poland).
The analysis of the obtained complete genomic sequences allowed the identification of different types of MGEs and provided valuable data on their structure and genetic load, with a particular focus on genes of adaptive value. In addition, we identified all ARGs present in bacterial chromosomes and defined their putative genetic carriers.
These analyses have led to the identification of a novel resistance island and new variants of MGEs that may play an important role in the dissemination of resistance phenotypes.

P100 Induction of antibiotic resistance mechanisms in strains isolated from burn wounds in the environment of minimal inhibitory concentration of silver sulfathiazole salts

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The prevention of burn wound infections is one of the main cornerstones of burn treatment. A wide variety of antibiotics are used in therapy. The most commonly used are topical bactericides and disinfectants in the form of dressings with the use of silver ions, silver sulfathiazole or octenidine. Despite good antimicrobial properties, silver sulfathiazole salt preparations used in the treatment of burns may cause some adverse patient side effects on the microbes. This phenomenon occurs when the silver salt of sulfathiazole is rinsed out along with the dressing exudate, which leads to the formation of concentrations below the MIC in the wound environment, which is a stress factor for microorganisms.

The aim of the study was to demonstrate the effect of the preparation used in Poland – silver salt of sulfathiazole on the induction of antibiotic resistance of the tested strains.
The experiment consisted in determining the minimum inhibitory concentration (MIC) of silver sulfathiazole and inducing resistance mechanisms through stress on *Staphylococcus aureus*, *Escherichia coli and Pseudomonas aeruginosa* in continuous culture at a defined concentration of sulfathiazole silver salt.
E. coli became antibiotic resistant the fastest whereas *S. aureus* was the least susceptible to induction of resistance mechanisms.
In microorganisms colonizing a burn wound, resistance mechanisms are gradually induced as the treatment progresses. The silver salt of sulfathiazole is an active inducer of antibiotic and chemotherapeutic resistance mechanisms.

Sulfonamide resistance via antibiotic inactivation and transmission among Gram-negative bacteria in municipal activated sludge

P101

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The spreading of antimicrobial resistance in environments, especially the transfer into human pathogens, has emerged as a global concern. A culprit for the rise of antimicrobial resistance issues is the misuse and/or unrestricted disposal of antibiotics, conducive to the prevalence of these persistent compounds that render the dissemination of antimicrobial resistance genes (ARGs). This is worse at municipal wastewater treatment plants (WWTPs) where antibiotics frequently occur at relatively high concentrations. In the present study, we focused on characterizing sludge microorganisms that are capable of inactivating antibiotics and investigating their molecular foundations. Sulfamethoxazole (SMX) was selected as a representative for sulfonamides (SAs), a class of synthetic antibiotics that have been extensively used. We successfully isolated two SMX degraders, *Methylophilus* sp. RD1 and *Xanthobacter* sp. LD2, which can cleave the S-N bond and subsequently dismiss the antimicrobial effort of SMX. Genome sequencing by Nanopore MinION revealed the presence of *sadA/sulX* genes that encode the class D FMNH2-dependent monooxygenases, known for their ability of catalyzing the breakdown of S-N linkage in SAs. This is the first report revealing the occurrence of *sadA/sulX* genes in Gram-negative bacteria prevailing in diverse environments. Phyletic patterns of these antibiotic inactivation genes and surrounding mobile elements provided molecular evidence for horizontal gene transfer (HGT), processes. Mating experiments also proved the occurrence of HGT particularly with the selective pressure of SMX. The identification of antibiotic inactivation genes and the associated molecular processes enables us to design efficient methods to eliminate antibiotics and thus decelerate the dissemination of antibiotic resistance.

P102 Multidrug resistant *Chryseobacteria* and their health implications

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Chryseobacteria consist of important human pathogens that can cause a myriad of nosocomial infections. We investigated 215 genomes of *Chryseobacterium* isolates available in the public databases and those isolated in our lab from activated sludge samples collected at domestic wastewater treatment facilities. A majority of these *Chryseobacteria* harbor 3 or more antibiotic resistance genes (ARGs) with the potential to confer resistance to at least 2 types of commonly prescribed antibiotics. The most abundant ARGs include beta-lactam class A (blaCGA-1 and blaCIA), and class B (blaCGB-1 and blaIND), and aminoglycoside (aadS, RanA, and RanB). These ARGs are considered potentially intrinsic given their similar GC contents to core genes and lack of mobile genetic elements (MGEs) in close proximity. A phylogenetic analysis based on universal marker genes and ARG profiles revealed no distinctive divergence among the isolates from environmental, animal, and clinical origins, suggesting the close relevance of clinical and environmental isolates. However, catB encoding chloramphenicol acetyltransferases that inactivate phenicols is highly enriched in clinical isolates, particularly collocated with the tetracycline resistance gene *tetX* and two MGEs, IS91 family transposase and tyrosine-type recombinase XerD. These genes associated with the adjacent sequences to form a resistance island that is firstly reported. This resistance island is mobile among bacteria in the *Bacteroidota* phylum, particularly those with high pathogenicity. Furthermore, *Chryseobacteria* exhibited global ubiquity in activated sludge samples and low susceptibility to disinfection, increasing the chance to bypass the WWTPs and contaminate the receiving water bodies, which poses imminent threats to human health and natural biota.

P103 How to combat antimicrobial resistance with limited resources: Kosovo case

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AMR (antimicrobial resistance) remains a significant challenge for health care in Kosovo, with resistance rates of all microorganisms being 2–5 fold higher than average in the EU countries. The aim of this study was to present the achievements and challenges of AMR in Kosovo. This review addresses challenges and current government initiatives to combat AMR in Kosovo. Wholesales data on antibacterial use in Kosovo was 26.3 DID (defined daily doses/1000 inhabitants/day) in 2011 and decreased to 20.1 DID in 2018. In all country hospitals, 56.8% of inpatients used at least one antibiotic, with ceftriaxone as the most prescribed. A prescription with

generic names was noted only in 31% of cases in primary care. Covid-19 was a significant accelerator of antibiotic misuse, particularly in the primary health care system.
The Ministry of Health in Kosova completed two national action plans for AMR. Laboratory investments were highest in the last two decades. Budget alternatives through open call grants were a significant response to budget constraints. The WHO approach AWaRe (Access Watch and Reserve) was introduced within the New Essential Medicine List of the Ministry of Health. The Faculty of Medicine has included the AMR as a new elective module at the undergraduate level, whereas translated educational package "E-bug" was launched for school children.
Key responses to combat AMR are the implementation of the "One Health" approach, increasing hand hygiene and immunization coverage, empowering laboratory capacities, raising awareness, prudent use of antimicrobials, infection prevention and control, and promotion of research and international cooperation.

Application of radiant catalytic ionization (RCI) in slurry and municipal sewage hygienization

P104

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Slurry and sewage can constitute a reservoir of numerous pathogenic microorganisms, including bacteria, fungi, microalgae and parasites and therefore pose a health risk to humans and animals. The aim of the study was to evaluate the hygienization effectiveness of fine bubble aeration with the use of RCI-activated air against selected bacteria, molds and parasite eggs in comparison to conventional atmospheric air aeration. The research included the enumeration of *Escherichia coli*, *Salmonella* senftenberg W775, *E. faecalis*, *Listeria monocytogenes*, *Clostridioides difficile*, *Aspergillus niger* and *Ascaris suum* invasive eggs in slurry and sewage of different densities. The microorganisms were introduced into the tested liquids in the form of suspensions (bacteria and fungi) or in special carriers (*A. suum* eggs) and for 12 days they were subjected to fine-bubble aeration (obtained concentration of dissolved oxygen: 1, 2 and 3 $gO_2 \times m^{-3}$) in variants without and with using RCI. Samples taken at 0, 1, 2, 4, 6, 8, 10 and 12 days were analyzed using appropriate microbiological media. The invasiveness of *A. suum* eggs was determined with microscopic observation. The lines of regressions were plotted and the theoretical survival times of the studied microorganisms were calculated. The shortest theoretical survival time, 14–17 days, was demonstrated for *L. monocytogenes* aerated with RCI-activated air (3 $gO_2 \times m^{-3}$, thick slurry), while the longest, exceeding 70 days, for *C. difficile* in the experiment without RCI (1 $gO_2 \times m^{-3}$, thin slurry). The applied hygienization method did not significantly differentiate the theoretical survival time of *A. niger* and the invasiveness of *A. suum* eggs.

P105

Correlation between antibiotic resistance level and soil physicochemical properties

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Dissemination of multidrug-resistant bacteria into the environment is considered as one of the most important global health problems. According to the World Health Organization (WHO), tackling antimicrobial resistance (AMR) requires an urgent action and should involve a holistic approach termed "One Health", which indicates the connection and interaction of three spheres: human, veterinary and environmental. In-depth investigation of the factors contributing to the resistance development, persistence and transmission of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) is necessary. The presented results are derived from a side study conducted as part of an international project entitled "ANTIVERSA – Biodiversity as an ecological barrier for the spread of clinically relevant antibiotic resistance in the environment" (National Centre of Science, grant no. 2019/32/Z/NZ8/00011).

Physicochemical properties and resistome of different soils in Poland were compared. Soil samples were collected from areas of high (fertilized and unfertilized fields, organic soils) and low (forests) anthropogenic activity and subjected to analysis of their physicochemical properties. Samples were tested for 27 ARGs and 5 MGEs (mobile genetic elements) of clinical relevance using Resistomap SmartChip qPCR system. Relationships between physicochemical properties of soils and ARGs were determined using Spearman's correlation coefficient.

Physicochemical parameters including pH, material content, nutrients and trace elements abundance correlated positively or negatively with some ARG ($|r|>0.7$, $p<0.05$). Our study suggests the existence of a strong relationship between ARGs and physicochemical properties of the examined soils. Larger scale research is required, taking into account nutritional requirements of specific bacteria types, including pathogens.

Evaluation of conventional methods versus whole-genome sequencing and MYCOTBI for the detection of resistance to rifampicin and isoniazid in *Mycobacterium tuberculosis* complex strains from Lithuania and Poland

P106

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Rapid and accurate rifampicin (RIF) and isoniazid (INH) resistance detection is essential for the treatment of multi-drug-resistant tuberculosis (MDR-TB). The objective of this study was to evaluate the performance of phenotype- and genotype-based drug susceptibility testing (pDST/gDST) methods for detection of RIF/INH resistance in relation to whole genome sequencing (WGS).

The study included 185 *Mycobacterium tuberculosis* isolates (124 MDR-TB; 61 drug-susceptible), recovered from patients in Lithuania (n = 122) and Poland (n = 63) (2018–2021). DST was performed using BACTEC MGIT-960 System and Löwenstein-Jensen (LJ) medium and MICs were determined using MYCOTBI plates. Line probe assay (LPA) was used for rapid resistance prediction. WGS was done with Illumina NovaSeq 6000 sequencer, and data were analyzed using either (I) bioinformatic pipeline or (II) freely available software platforms (Mykrobe, TB Profiler). The congruency of BACTEC/LJ with LPA was higher than with pipeline-based WGS results (98.9% [RIF]; 97.8% [INH] vs. 97.3% [RIF]; 97.3% [INH]). MIC ranges for genotypically susceptible isolates were ≤0.12–0.5 µg/ml and ≤0.03–0.06 µg/ml for RIF and INH, respectively. The most prevalent *rpoB* mutation was S450L (79.8%), and the majority of mutants demonstrated MICs of >16 µg/ml. *katG* S315T (85.3%) mutants and those with an extra *inhA* C-15T (12.1%) or *inhA* T-8A (0.8%) mutation had MICs within the range of 0.12–>4.0 µg/ml. Single *inhA* C-15T mutant had an MIC of 1 µg/ml.

The overall discordance rate between pDST and gDST results was 3.8%. Five isolates were found that displayed unexplained discordant results. This may relate to heteroresistance, mixed infection or methodological flaws.

P107 Comparison of phenotypic and molecular drug resistance testing of *Mycobacterium tuberculosis* strains isolated in Poland

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[2] Biobank Lab, Department of Molecular Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Poland
[3] Department of Microbiology, National Tuberculosis and Lung Diseases Research Institute, Warsaw, Poland
[4] Laboratory of Genetics and Physiology of *Mycobacterium*, Institute of Medical Biology, Polish Academy of Sciences

Drug-resistant tuberculosis is a major threat to patients worldwide due to costly and ineffective treatment. Traditional drug susceptibility testing (DST) is simple but time-consuming, hence molecular analysis thru next-generation sequencing may be a key to faster diagnostics. The aim of the study was to compare phenotypic and molecular DST on a population of M. tuberculosis outbreak strains isolated in Poland in years 2010-2021.

We analysed 303 strains of Mycobacterium tuberculosis. We tested their drug susceptibility for isoniazid (INH), rifampicin (RMP), ethambutol (EMB), and fluoroquinolones (FQ) with BACTEC MGIT 360 system using standard protocol from the manufacturer. Whole genome sequencing was performed with Illumina's NextSeq 500. The identification of specific variants in genes associated with drug resistance was performed with MTBseq v. 1.0.3. The results were manually curated, obtained point mutations were evaluated using WHO catalogue of mutations of drug resistant mutations. Phenotypic susceptibility testing was considered a reference method for the analysis.

Traditional DST revealed 75 drug resistant strains and 228 drug susceptible strains. Molecular DST identified 90 drug resistant strains. Both methods of DST agreed in 41.4% of strains for FQ, in 78.6% for RMP, in 76% for INH and in 38.9% for EMB.

Our results suggest that the use of NGS may be effective in the diagnostics of drug resistance in M. tuberculosis strains circulating in Poland for RMP and INH, but it may be less efficient for FQ and EMB.

Funding: NCN research grant OPUS 18 no. 2019/35/B/NZ7/00942.

The Royal Castle in Warsaw.

Photo by Filip Kwiatkowski © City of Warsaw

VIII. Miscellanea

P108 The role of bacteria and neutrophils in pathogenesis of oral mucositis

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Oral mucositis (OM), characterized by a massive inflammation and ulceration of oral cavity, is a common complication of anti-cancer treatments. Untreated, OM severely decreases quality of life, and can become life-threatening if it prevents oral nutrition and hydration, or if it becomes an entry for systemic spread of oral bacteria. The exact pathophysiology of OM is not known, but it has been thought to result from chemotherapy-induced death of oral mucosal cells and from excessive local inflammation, driven by macrophages and incoming neutrophils. By using antibody-mediated depletion in a mouse model of OM, we demonstrated that contrary to their presumed pro-inflammatory role, the neutrophils play in fact a beneficial role in OM. In the absence of neutrophils ulcer healing is delayed inflammation increases, and bacteria invade the surrounding deeper tissues. Surprisingly, these beneficial effects of neutrophils were not directly linked to their anti-bacterial activity, as prevention of bacterial entry into tissues with antibiotics had no protective effect, and as ulcer healing correlated with circulating neutrophil levels, but not with the local bacterial tissue load.

Activity of selected oxidative enzymes (GST, R-GSSG and GSH) in platelets in patients with COVID-19 during one-month observation

P109

Małgorzata Goszka[1]*, Patrycja Stodolak[1], Aleksandra Polikowska[1], Elżbieta Cecerska-Heryć[1]

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In response to oxidative stress developing during various diseases, including COVID-19, cells have produced an antioxidant defense system consisting of, e.g. antioxidant enzymes.
The aim was to determine the activity of selected oxidative stress biomarkers in COVID-19 patients and medical workers with acquired immunity.
86 subjects (31 health workers with acquired immunity to COVID-19 and 55 patients with COVID-19) from whom blood was collected four times (1, 7, 14, 28 days after detection of Ig+ antibodies) were qualified for the study group. The control group (NK) consisted of 56 healthy volunteers who did not have increased levels of COVID-19 antibodies. The antioxidant enzyme activity was determined in PRP (platelet-rich-plasma) using spectrophotometric method.
Statistical analysis has shown a significant relationship between the activity/concentration of GST, R-GSSG and GSH ($p < 0,001$; $p < 0.001$, $p = 0.004$) between our study groups and the control group. The relationship between GSH concentration was found in people who died from COVID-19 and those who survived the disease ($p = 0.049$). GSH levels were significantly lower among people who died.
Patients suffering from COVID-19 are exposed to severe oxidative stress, evidenced by much higher GST and R-GSSG and GSH activities/ concentration than the control and IG+ groups. This stress is not compensated even after 28 days from the detection of the disease, shown by the activities of GST and R-GGSG in the fourth collection, which are still increased. Low GSH levels predisposed to death from COVID-19.

P110 The effect of rotating magnetic field exposition on lytic bacteriophage life cycle

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[2] Department of Chemical and Process Engineering, Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology in Szczecin, Poland

Electric and magnetic fields influence basic life processes of microorganisms. Electromagnetic fields affect enzyme activity, cell viability, metabolic activity, and proliferation rate. Additionally, DNA synthesis, transcription, and translation processes can be overregulated. Using the rotating magnetic field (RMF) to enhance and support biotechnological processes is promising. At this moment, there are no field-based methods for the support and intensification of bacteriophage production. This study aimed to evaluate the influence of RMF on the selected bacteriophage properties.

The obtained data showed that RMF modified the bacteriophage lifecycle and lytic activity. It was observed that RMF exposition shortened the latent period of the lytic cycle of T4-phage from 20 min to 15 min (excluding the adsorption time). The burst size significantly increased from 103 PFU per infected cell to approx. 330 PFU per infected cell. Additionally, the study showed an increased lysis activity of this phage on liquid cultures of *Escherichia coli* when temporary (1h) exposition was used. These experimental results showed the potential usage of RMF in bacteriophage research and process bioengineering.

This study was supported by National Science Centre Poland within the PRELUDIUM 15 Programme [2018/29/N/ST8/01043].

P111 Search for plant species with antimicrobial activity

Anastasia A. Hermosaningtyas[1*], Barbara Thiem[1], Jaromir Budzianowski[1], Małgorzata Kikowska[1]

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The crisis of antimicrobial resistance has been on alert around the world. According to the Global Antimicrobial Resistance and Use Surveillance System Report released by the World Health Organization (WHO) in 2021, the median resistance rate to the third generation of antibiotics reached 25–50%. The efforts to discover new antimicrobials are still in play, and natural sources have a promising future. Medicinal plants have been used to cure infections since ancient times and are still widely used in ethnomedicine. Plants contain an abundance of secondary metabolites beneficial for antimicrobial, such as phenolic acids, flavonoids, tannins, terpenoids, and alkaloids. These metabolites are unique, and some have complex structures that are hard to be synthetically made. Phytochemicals can disrupt microbial membranes, impair cellular metabolism, disrupt biofilm formation, or enhance the therapeutic effect of antibiotics.

Our laboratory conducted studies to evaluate the antimicrobial activities of selected species (*Solidago virgaurea* L., *Rubus chamaemorus* L., *Lychnis flos-cuculi* L., *Eryngium planum* L., *E. campestre* L., and *E. maritimum* L.) which grow naturally or are from *in vitro* cultures. The alcoholic extract and the fraction of selected species possess antimicrobial properties. *L. flos-cuculi* has antifungal, antibacterial, and anti-amoebic properties. *Eryngium* spp. has vigorous antimycotic activities but moderate antibacterial activities. *R. chamaemorus* is active against some Gram-positive bacteria, and *S. virgaurea* showed moderate bactericidal properties. Our studies highlight the importance and capability of plant extracts/compounds as new and promising antimicrobial agents.

The role of NAD^+ binding in HopAG1 virulence P112

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Pseudomonas syringae is a Gram-negative, world-widely distributed bacterium, which is one of the most frequent causal agent of bacterial diseases devastating plants, including important crops and fruit trees. The success of *P. syringae* as a pathogen depends on a wide range of effectors delivered into plant cells *via* type three secretion system.

Studying the function of particular effectors may help understand molecular mechanisms of host-pathogen interactions, which may be useful in developing new methods of plants protection. Effectors usually target molecules and processes essential for plants, which are often evolutionarily conserved among kingdoms. This suggests that results obtained in plant-bacterial field might be surprisingly broadly applicable.

The aim of our project is to determine the role of an uncharacterized *P. syringae* effector – HopAG1.

Bioinformatic analysis revealed that HopAG1 might contain three functional domains: ADP-ribosyltransferase (ART), kinase and Nudix hydrolase. ARTs catalyse ADP-ribosylation – a post-translational attachment of ADP-ribose originated from NAD^+. It is also known that NAD^+-derived molecules activate immune signalling and it was recently shown that some pathogens are able to manipulate host NAD^+ metabolism.

To investigate NAD^+ involvement in HopAG1 virulence, variants of the effector with substitutions within the predicted NAD^+ binding site were expressed in *N. benthamiana*. In contrast to wild-type HopAG1 causing tissue collapse, the variants impaired in NAD^+ binding showed compromised disease symptoms, as well as changed subcellular localization. These observations suggest that NAD^+ binding is crucial for the function of the effector.

The work was supported by National Science Centre (2018/31/D/NZ3/03296).

P113 Microalgae phosphorus starvation for tertiary wastewater treatment

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Conventional wastewater treatment typically reduces nutrients to levels acceptable by national and international regulations. Simultaneously, these norms are not legally binding for small scale wastewater treatment systems (< 2 kPE). The suggested technologies for tertiary treatment in small scale WWTPs are consuming large areas, e.g., slow-rate overland flow, slow-rate subsurface infiltration, and not allowing to recover phosphorus (e.g. sorbents). Subsequently, there is still a need for suitable technologies and microalgae-based treatment is one of the potential suggestions since it both effectively removes nutrients and produces valuable biomass. Furthermore, it is estimated that algal biomass exposure to phosphorus starvation conditions can enhance the phosphate uptake from municipal wastewater. Within this research we aimed to estimate the optimal starvation conditions to develop sustainable and practically applicable technology. Microalgal biomass was exposed to phosphorus deficiency conditions for periods varying between 1 and 10 days and inoculated at different initial biomass and phosphate concentrations. A 10-day period of phosphate deficiency, supported by low initial biomass concentration (~0.25 mg DW L^{-1}), increased the phosphate removal by 62–175% when compared to the reference conditions. A 10-day period of biomass P-deficiency also boosted the polyphosphate accumulation and protein productivity, increasing them up to 40 and 46.8 times, respectively compared to reference conditions. The obtained results present microalgae exposure to phosphorus stress as a supplementary tool for wastewater post-treatment targeted at rapid phosphorus removal.

Acknowledgement: This study was supported by the project “Post-treatment of municipal wastewater using sequenced-batch photobioreactor technology” (Project No. LZP-2019/1–0271).

P114 Bacteriophage activity against *Staphylococcus aureus* in a mucin environment and a cosmetic product based on snail serum

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Staphylococcus aureus is a common etiological factor in skin infections. Phages are naturally obligate parasites of bacteria and can interact with their hosts. The research presented here aimed to choose the right concentration of phage and serum to make the therapy the most effective. 24-hour lysis of *S. aureus* in liquid culture was performed by adding snail serum (100%–0.2%) and vB_SauM-A phage (109–102) at different concentrations. Then, *S. aureus* viability and phage adsorption for 4 hours (1-hour intervals) were determined in the solutions (containing specific concentrations of serum, phage, or serum +phage). After treatment, *S. aureus* was inoculated,

and phages were purified and titrated. To determine the appropriate concentration of mucins, adsorption of bacteriophage (time intervals:1, 5, 10, 15) in mucin medium with different concentrations (0.5%, 1%, 1.5%, 2.5%) was also carried out.
The results show that among all possible combinations, phage (concentration of 106) is the most active in serum at a concentration of 3.125%. The content of mucins, both type II and III, and those derived from snails was observed to change the parameters of bacteriophage adsorption to the host cells. The binding of bacteriophages to mucins was also observed. The occurring phenomena change the parameters of lysis and the effectiveness of bacteriophages.
In conclusion, the activity of bacteriophages in the environment with mucins is higher and necessary for the successful elimination of *S. aureus* from liquid culture. The presented aspect should be considered when planning alternative therapies based on the synergistic action of these products.

Changes in the activity of antioxidant enzymes (SOD, CAT, GpX) in platelets in COVID-19 patients during one-month observation

P115

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Antioxidant enzymes are considered oxidative stress biomarkers in many diseases including COVID-19.
The aim was to investigate the exposure of COVID-19 patients and health workers with acquired immunity to oxidative stress.
86 subjects – 31 health workers with acquired immunity to COVID-19, 55 subjects with COVID-19 – patients, from whom blood was collected four times (in 1, 7, 14, 28 days after Ig+ antibodies detection) were qualified for the study group. The control group (NK) consisted of 56 healthy volunteers who did not have elevated levels of anti-COVID-19 antibodies. The antioxidant enzyme activity was investigated in PRP (platelet-rich-plasma) by spectrophotometric method. Statistical analysis has shown a significant relationship between the activity of CAT, SOD and GPx ($p = 0{,}001$; $p < 0.001$, $p < 0.001$) between study groups and the control group, and between all COVID-19 patients (including asymptomatic IG+ group) ($p < 0.001$, $p < 0.001$, $p < 0{,}001$). The relationship was found between SOD activity in people who died from COVID-19 and those who survived. ($p \geq 0.049$). Patients who died 14 days after the onset of the disease had high CAT activity before death ($p = 0{,}0147$).
Much higher SOD and GPx activities in COVID-19 patients than the control and IG+ groups suggest severe oxidative stress caused by this acute syndrome. This stress is not compensated even after 28 days from disease detection, as evidenced by the gradually increasing activities of the tested enzymes in the tested groups. Among the people who died in the course of the studies, high catalase activity was a negative biomarker of survival.

P116 Developing an antiseptic, analgesic and anti-inflammatory herbal and vegan oral spray and researching its antimicrobial activities against various bacteria, fungi and viruses

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[2] Kurtsan İlaçları A.Ş. Istanbul, Turkey

Since the mouth and throat are the most important entrances for pathogens, using nasal and oral antisepsis would be beneficial to fight upper respiratory tract infections in particular. In this study, an oral spray with antiseptic, analgesic and anti-inflammatory properties was developed, and formulated with completely natural and vegan/herbal content, and its antiviral, antibacterial, and antifungal activities were tested compared with the active ingredients in the formulation.
The minimum inhibitory concentrations (MIC), time-kill curves (TKC), and antiviral activities of oral spray and its active ingredients were determined against mouth and upper respiratory tract pathogens group A beta hemolytic *Streptococcus* (GABHS) clinical isolate, *Streptococcus pyogenes* ATCC 19615, *Staphylococcus aureus* ATCC 29213, *Klebsiella pneumoniae* ATCC 4352, the yeast *Candida albicans* ATCC 10231, and parainfluenza virus type-2.
All studied herbal substances showed MICs ranging from 0.4% to 25%, depending on the microorganism, while those for formulation were 0.4–3%. TKC analysis showed oral spray has strong –cidal activities (≥3-Log_{10}) against all studied bacteria and yeast, except *S. aureus*, starting at 2th hour, and reaching maximum activity at 4th hour. The oral spray and the active ingredient mixture have strong antiviral activity against parainfluenza virus type-2, (78% and 68%, respectively).
According to our findings, the formulated and developed oral spray has several advantages such as having antiviral, antibacterial, and antifungal activities, being completely natural and vegan, free of chemical antiseptics, creating a moisturizing and soothing protective layer with a barrier. The ease of use of the spray form is also of importance.

City View from Palace of Culture and Science, Warsaw.

Photo by Tomasz Nowak © City of Warsaw

Polish Thread in the Biography of Louis Pasteur

The Polish Disciple of Louis Pasteur – Odo Bujwid (1857–1942)

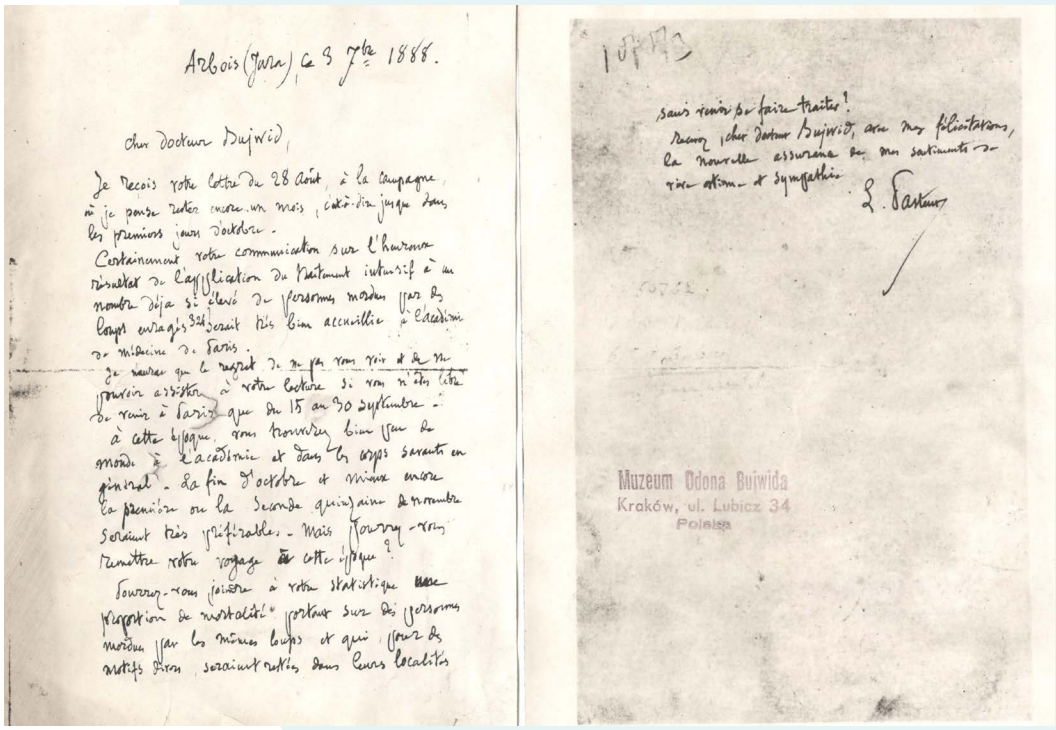
Dominika SALAMON
Jagiellonian University Medical College, Krakow, Poland

Odo Bujwid, who is called the father of Polish microbiology, graduated from medical school in Warsaw. Improving his work with the microscope under the supervision of the famous histologist, Professor Henryk Hoyer, his attention focused on bacteriology, which became the basis for the development and practical use of this field of knowledge. While still a student, he travelled to Berlin for a bacteriology course led by Robert Koch. A year later, in 1885, Bujwid opened the first bacteriological laboratory in Poland and organized courses for physicians. His acquaintance with

Odo Feliks Kazimierz Bujwid. Photograph from the collection of the Odo Bujwid Museum in Krakow (scan made by the Malopolska Institute of Culture, Krakow)

Louis Pasteur began in 1886, when the Polish bacteriologist travelled to the Pasteur Institute to get to know Pasteur and learn his methods of vaccination against rabies. He got 2 rabbits infected with rabies virus from the French scientist, which allowed him to embark on fighting this disease in Poland. After returning to Warsaw, he adapted his studio to create the world's second vaccination station according to the Pasteur method. It was a bold step, because at that time the world of science, including Robert Koch, strongly criticized the vaccination method developed and performed by Louis Pasteur. Perhaps this attitude of Bujwid, as well as his observations, research and energetic activities in the field of vaccinology and microbiology, determined the later, close relationship of both scientists.

In 1893, he moved to Krakow, where he became a professor at the Department of Hygiene at the Jagiellonian University and studied air, water and food. The Polish bacteriologist also worked on tuberculosis (it was Odo Bujwid, who called the substance from Robert Koch "tuberculin"), cholera, plague, typhus, anthrax, malaria, as well as diphtheria and tetanus (serum production). His broad interests, pioneering microbiological research, as well as experiences on tropical diseases brought from a trip to South America made him a well-known and respected physician and scientist in Poland. 2022 is the 80th anniversary of his death.


Arbois (Jura) ce 3 7bre 1888.

Cher Docteur Bujwid,

Je reçois votre lettre du 28 Août, à la campagne où je pense rester encore un mois, cette fois jusque dans les premiers jours d'octobre.

Certainement votre communication sur l'heureux résultat de l'application du traitement intensif à un nombre déjà si élevé de personnes mordues par des loups enragés sera très bien accueillie à l'Académie de Médecine de Paris.

Je saurais gré le regret de ne pas vous voir et de ne pouvoir assister à votre lecture si vous n'êtes libre de venir à Paris que du 15 au 30 septembre.

À cette époque, vous trouverez bien peu de monde à l'Académie et dans les corps savants en général. La fin d'octobre et même encore la première ou la seconde quinzaine de novembre seraient très préférables. Mais pourrez-vous remettre votre voyage à cette époque?

Pourrez-vous joindre à votre statistique une proportion de mortalité? Pasteur sur des personnes mordues par les mêmes loups et qui pour des motifs divers seraient restées dans leurs localités

sans venir se faire traiter?

Recevez, cher Docteur Bujwid, avec mes félicitations, la nouvelle assurance de mes sentiments les plus affectueux et sympathiques.

L. Pasteur

Muzeum Odona Bujwida
Kraków, ul. Lubicz 34
Polska

One of the letters of Louis Pasteur (dated September 3, 1888) to Odo Bujwid, in which the French scientist regrets that he will not be able to attend the speech of the Polish microbiologist in front of the representatives of the Paris Academy. O. Bujwid is to present his results of treatment of people bitten by rabid animals. Pasteur is very curious about Bujwid's observations, but he is currently in the countryside (Arbois – Pasteur's hometown) and plans to return only at the end of October. He invites the Pole to come again to Paris in mid-November, because then both men could meet. Under the signature of L. Pasteur, the seal of the now closed, private museum of Odo Bujwid in Krakow, at 34, Lubicz Street (Poland) is visible. The letter comes from the collection of the Odo Bujwid Museum in Krakow (scan made by the Malopolska Institute of Culture, Krakow).

The Palace on the Isle in the Royal Baths in Warsaw.

General Information

Conference Venue

Copernicus Science Centre

The Copernicus Science Centre is an exceptional, already iconic, institution of culture, whose ultra-modern building stands on the west bank of the Vistula river, in the quaint downtown district of Powiśle. The Centre is the largest science museum in Poland and one of the most advanced in Europe. It provides over 450 audio-visual and interactive exhibits, which allow visitors to carry out experiments on their own and delve into the mysteries of nature in a witty and playful way.

All participants of the conference can visit the museum exhibition with the tickets included in the conference materials.

Copernicus Science Centre
Wybrzeże Kościuszkowskie 20, 00-390 Warsaw
https://www.kopernik.org.pl/en

You can reach Copernicus Science Centre with:

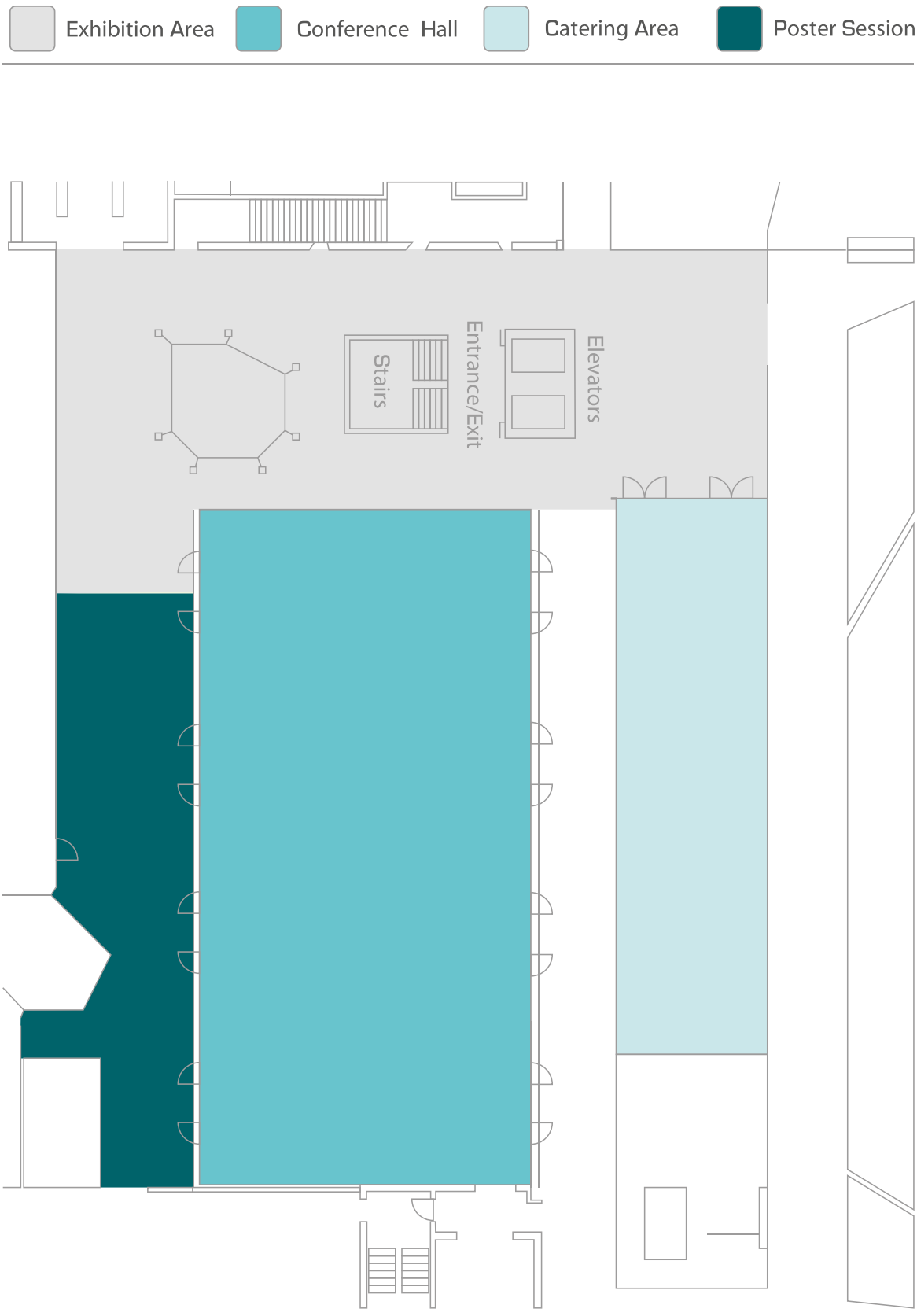
- M2 metro line – "Centrum Nauki Kopernik" station

or the following bus lines:

- 106, 118, 127 – "Biblioteka Uniwersytecka" bus stop
- 185 – "Metro Centrum Nauki Kopernik" bus stop (in the tunnel)
- 102, 162 – "Metro Centrum Nauki Kopernik"

Warsaw Chopin airport is only 30 minutes away by car.

The Copernicus Science Centre, Warsaw.



Conference Venue Overview

Special Events

Speakers & Guests Welcome Reception

The Welcome Reception will be held on the 28th November at The Columned Hall of the Faculty of History, University of Warsaw. The Columned Hall, decorated with statues from the collection of the former Plaster Cabinet of the University of Warsaw, is among the most beautiful classical interiors in Warsaw.
Among the splendour of authentic, historical statues and malachite columns, many outstanding individuals used to walk through the Hall, including Fryderyk Chopin. Today, the Columned Hall holds the representative function for the University of Warsaw.

The Faculty of History is located on the Main Campus of the University of Warsaw. Since 1938 the Faculty has been housed in the Former Museum Building. This classicist building was built between 1818–1820, according to the project of Michal Kado. It is the only original building in Warsaw in the Warsaw Classical style that was not destroyed during World War II. In the years 1820–1831, the building housed the Fine Arts Department of the University of Warsaw.

Piano Concert

During the Welcome Reception, we cordially invite our Guests to listen to the piano concert given by Wojciech Świętoński.

Wojciech Świętoński graduated from the Chopin University of Music in Warsaw in the piano class of Professor Bronisława Kawalla. He is one of three participants in Maestro Ivo Pogorelich's elite 'Master Class'. Laureate of international piano competitions in Kiev and Rome. He regularly performs in the concert halls of Europe, in recitals and with orchestral accompaniment. He has collaborated with many ensembles, including Polish Sinfonia Iuventus Orchestra, Polish Radio Orchestra, National Polish Radio Symphony Orchestra. He actively performed during the celebrations of the Year of Karol Szymanowski and the Year of Fryderyk Chopin. His performances inaugurated the Schuman Parade and the opening of the Fryderyk Chopin Museum in Żelazowa Wola.

Participation is upon invitation only.

L'Oréal-UNESCO For Women in Science Gala

The L'Oréal-UNESCO For Women in Science scholarship program is a unique project addressed to Polish scientists. The aim of the program, carried out in Poland since 2001, is to promote the scientific achievements of talented researchers, encourage them to continue their work aimed at the development of science, and provide them with financial support. The program partners are the Polish UNESCO Committee, the Ministry of Education and Science, the Polish Academy of Sciences and the UN Global Compact Network Poland. Scholarships are awarded in three categories: master's, doctoral and postdoctoral based on the evaluation of an independent jury chaired by prof. dr hab. Ewa Łojkowska. The jury consists of scientists representing various fields of science from the largest academic centers in Poland. So far, 111 scientists in Poland have received L'Oréal-UNESCO For Women in Science scholarship.

L'Oréal-UNESCO For Women in Science Awards Ceremony, June 23rd 2022, Paris, France.

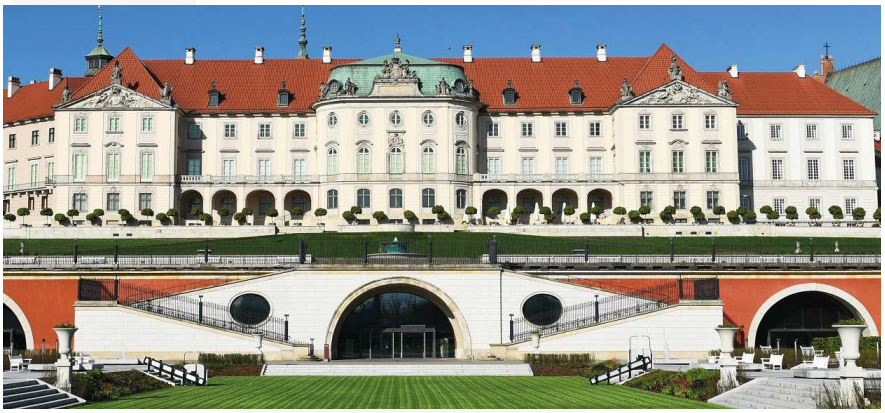
Poland is one of 118 countries in which scholarships for talented female researchers are awarded each year. The Polish edition of the For Women in Science Program is part of the global initiative, which was created thanks to the partnership between L'Oréal and UNESCO. The fellows of the national editions have a chance to receive international awards: the International Rising Talents award, which is given annually to 15 women. There are already four Polish women among them: Bernadeta Szewczyk – 2016, Joanna Sułkowska – 2017, Agnieszka Gajewicz – 2018, Karolina Mikulska-Rumińska – 2022).

On November 30th, 2022, the winners of this year's 22nd edition of L'Oréal-UNESCO For Women in Science will be announced. During the ceremony, we'll meet 6 talented scientists who will join the prestigious group of scholarship holders.

Each year L'Oréal Fondation, together with UNESCO, awards 5 most outstanding women in science by granting the international L'Oréal-UNESCO For Women in Science Awards. The discoveries and research conducted by the winners respond to the greatest challenges facing the world today. Among them are five Nobel Prize laureates: Christine Nusslein-Volhard and Elizabeth Blackburn in medicine and physiology, and Ada Yonath, Emmanuelle Charpentier and Jennifer Doudna in chemistry.

Gala Dinner

The Gala Dinner will take place on the November 29th at the Royal Castle in Warsaw. The Dinner will be held in the Kubicki Arcades – the only World War II preserved part of the Royal Castle.



The Kubicki Arcades were built in 1818–1827, according to a design by Jakub Kubicki, an architect of the Classicist epoch. The Arcades connected two gardens of the Castle: the upper garden situated on the escarpment, and the lower one located in the area adjoining the bed of the Vistula River. The arcades were one of the very few parts of the Royal Castle to survive the destruction of World War II. In 1995–2009, the Arcades underwent a major renovation, becoming an excellent venue for celebrating exceptional events. (Photo by M. Kmieciński)

The evening will be highlighted by a performance by the famous "Mazowsze" Folk Song and Dance Ensemble, Poland's best recognized folk ensemble internationally.

The National Folk Song and Dance Ensemble "Mazowsze" is one of the largest artistic ensembles in the world. Established and inspired at its core by dances, songs, chants, and traditions of Poland's central region – Mazowsze, the ensemble's repertoire draws from the folklore of virtually all Polish regions. Today, the repertoire of "Mazowsze" includes popular folk songs from around the world. On foreign tours, they are sung in over 40 languages.

Reception at the French Embassy in Warsaw

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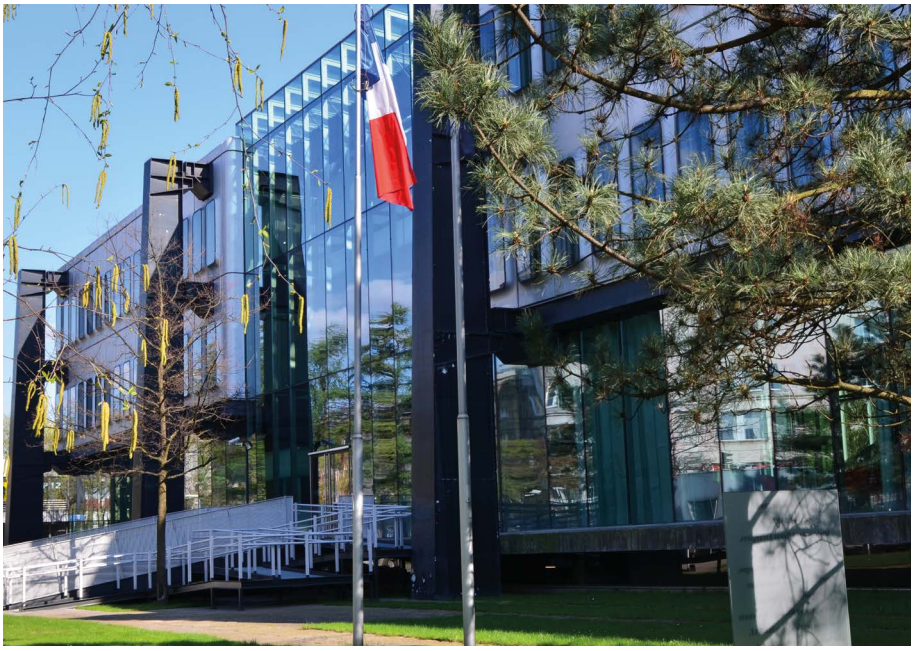
Frédéric Billet, Ambassador of France in Poland.

The patronage over the conference is held by The French Embassy in Poland.

French Ambassador to Poland, Frédéric BILLET, will give a welcoming speech at the opening of the conference, on November 29th.

A reception in honour of the conference for the 200th anniversary of the birth of Louis Pasteur will be held at the invitation of the French Ambassador. The reception will take place on the evening of November 30th, at the Embassy of France in Warsaw, Jazdów 9 street.

For security and capacity reasons, the number of guests is limited (participation upon invitation only).



Embassy of France, Warsaw.

The Best Abstract Award

The Best Abstract Award is sponsored by L'Oréal-UNESCO For Women in Science Programme. The awards, and certificates, will be given to the presenting authors of the best abstracts from each topic category. The winners will be selected by the conference scientific and organizing committees.

The Best e-Poster Presentation Award

This award is sponsored by *Microbiology Research*, an MDPI journal. It is designed to inspire speakers who are willing to share their research and insights in microbiology at "The Last Word Belongs to Microbes". There will be three winners of the award, each of whom will receive a prize and a certificate, and the winners will be selected by the conference scientific and organizing committees.

The Prize:

- First Prize: 250 CHF
- Second Prize: 150 CHF
- Third Prize: 100 CHF

Microbiology Research (ISSN 2036-7481) is an international and multidisciplinary scientific open access journal that publishes original research, review articles, editorials, perspectives, case reports, and brief reports to benefit researchers, microbiologists, physicians, veterinarians, and agronomists.

The main aim of *Microbiology Research* is to encourage researchers from diverse areas to publish theoretical and experimental results of research from all fundamental fields of microbiology in a one-health and circular health perspective, including applied microbiology. The full experimental procedure must be provided so that the results can be reproduced. There is no limitation on the length of articles for this journal.

About Warsaw

History

Warsaw, located on the River Vistula (WISŁA), is the capital of Poland and the largest city of the country with around 1.8 million residents, which makes it also the 8th largest city in the European Union. The history of the city begins in the 13th century, when it was officially founded by the Dukes of Masovia. The city began to flourish in the 16th century with the transfer of the capital from Krakow. The second heyday came with the Industrial Revolution of the 19th century. Before World War II, Warsaw was known for its elegant architecture and urbanism. Unfortunately, it was almost completely destroyed during the hostilities and nowadays is a mixture of districts re-constructed in myriad historical styles (e.g. the Old Town), and created after the War in socialist realism and contemporary architectural trends.

Warsaw is a major centre of business, science and education. Suffice to mention that the Warsaw Stock Exchange is the largest in Central and Eastern Europe and that the city hosts some of the finest institutions of higher education in Poland, including the University of Warsaw, Warsaw University of Technology, Medical University of Warsaw or Fryderyk Chopin University of Music. The total number of students in the city is approximately half a million, which is around 30% of the city population.

Local food

Polish cuisine is amazingly varied and rich in flavours. To start with, you should try a bunch of Polish soups. The most typical include sour rye soup (ŻUREK), served with white sausage and egg, sauerkraut soup (KAPUŚNIAK), and beetroot soup (BARSZCZ), usually accompanied with delicious pastries (PASZTECIKI) stuffed with cabbage or meat.

Classics among the main courses are dumplings (PIEROGI), dough wrappers with a variety of savoury or sweet fillings, hunter stew (BIGOS), made with boiled sauerkraut, spare ribs, and special seasonings or breaded pork chops (KOTLET SCHABOWY), best with potato purée and a mix of salads. Polish cuisine has also a large offer for vegetarians and vegans. Meat-free cuisine enjoys increasing popularity in Warsaw, and there are a lot of restaurants serving exclusively plant-based food.

As a dessert we recommend the traditional Polish doughnuts (PĄCZKI), typically with a fruit jam stuffing, which are freshly fried and sold still warm in every cake shop and bakery in the city.

Culture

Countless museums, art galleries, theatres, and cinemas provide extensive cultural services to residents and tourists in Warsaw. Among the places that are definitely worth visiting are the Museum of Warsaw, the POLIN Museum of the History of Polish Jews, the Warsaw Uprising Museum, the National Museum, and the Fryderyk Chopin Museum.

Social activities

Warsaw is full of cafes, bars, art zones, and trendy clubs. For social activities, check Poznańska street (especially near Wilcza street), and Plac Zbawiciela, or take a stroll through the historic streets of Praga District and visit Praga Koneser Centre. We also recommend the Elektrownia Powiśle Mall, which is a beautiful example of recent renovation of industrial zones in Warsaw.

Parks

There are over 70 parks in Warsaw, which makes it one of the greenest cities in Europe. Warsaw's Łazienki Park is one of the largest palace and park ensembles in Europe. Pole Mokotowskie is a large park located close to the city centre with a much more modern character. Królikarnia is a small, cosy park with a 20th century art gallery located in it. We strongly recommend the wild Vistula banks – a very rare example of natural spaces located in the centre of a European city – with a stunning view over the Old Town and the skyscrapers in the city centre.

Time

Poland is among the countries that use Central European Time (GMT + 01:00). This time zone applies to the majority of Europe, including Spain, France, Germany, Netherlands, Italy, Austria, Slovakia, Hungary, and many other countries.

Weather

The weather in Warsaw in autumn is relatively mild, with average highs of 10°C (49°F) and lows of 0°C (32°F). The average temperature is 3°C (37°F) in November. You can expect rain or even snow showers, but it may also be sunny and dry.

Language

The official language of Poland is Polish. Some important Polish phrases that might help you during your stay can be found at https://theculturetrip.com/europe/poland/articles/12-polish-phrases-you-need-to-know/. Polish may seem very complicated for foreigners, but you should have no problems communicating in English with local people, especially young and middle-aged Poles.

Currency

The national currency of Poland is the Polish ZŁOTY (zł or PLN) which equals 100 GROSZY (gr). Coins: 1, 2, 5, 10, 20, 50 gr, and 1, 2, 5 zł. Notes: 10, 20, 50, 100, 200, 500 zł. You can exchange your money at banks or exchange offices (KANTOR). The current exchange rates may be found at https://www.nbp.pl/homen.aspx?f=/kursy/ratesa.html%20. Note, however, that you should be able to pay using your standard debit card in most places.

Telephones

The area code for Warsaw is 22, whereas the country code is 48. When calling internationally to Warsaw from a mobile or a local phone unit, dial +48 22 or 22, respectively, followed by the rest of the number.

Emergencies

In case of emergency, call Police (997), Fire Brigade (998) or Ambulance (999) for help. When using a mobile phone dial 112.

Transport

Public transport in Warsaw is ubiquitous, serving the city with buses, trams, metro, and urban rapid rail lines. The easiest way to find the best transport between any two points in the city is to use website applications (e.g. https://jakdojade.pl/warszawa/trasa/). Ticket machines are located in most vehicles, near bus stops, and in metro stations. There are also several applications that allow you to buy one-way and short-term tickets. We recommend to buy a 3-day (72-h) ticket which costs 36 zł (ca. 8 euros) for Zone 1 (within the city borders). In the public transport in Warsaw the ticket must be validated only once and is also needed to pass the metro gates. (i.e. the same tickets are valid for buses, trams, and metro) To get onto the bus or tram you can use any door you like. For more information please visit at https://www.wtp.waw.pl/en/.

Opening hours

Most shops are open on weekdays from 10:00 to 19:00. On Saturdays they usually close at 14:00. The shops are closed on Sundays, except pharmacies (APTEKA), small groceries, and gas stations.

Postal services

Most post offices (POCZTA) are open from 8:00 to 20:00. Stamps, used on letters or postcards, can only be purchased at post offices.

More information

If you are interested in further information about important sightseeing spots in Warsaw, check the very well prepared tourist information website of the city (https://warsawtour.pl/en/warsaw-tourist-information/).

Night view of the city and Vistula River, Warsaw.

Photo by Filip Kwiatkowski © City of Warsaw

Commemorations

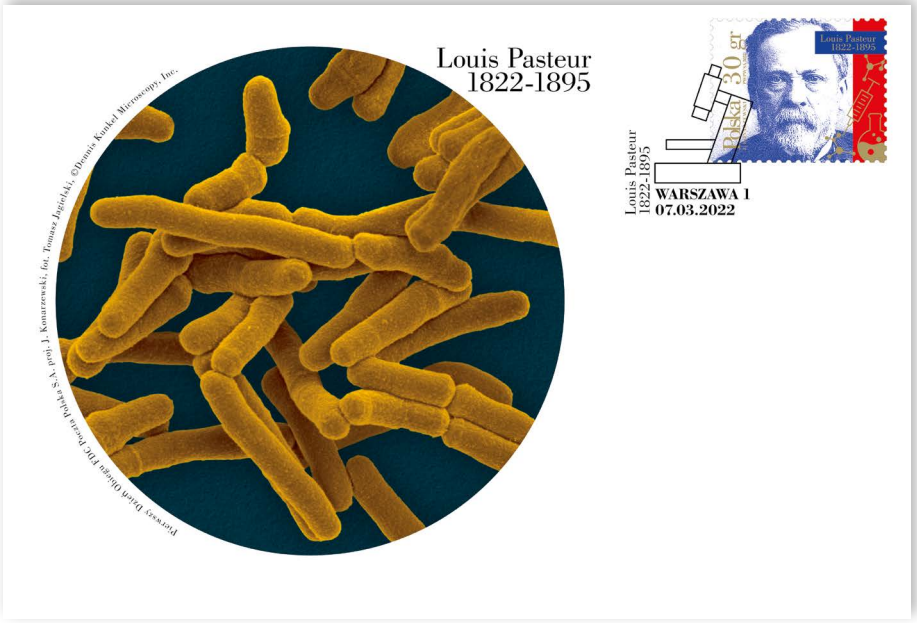
To commemorate the 200th anniversary of the birth of Louis Pasteur, the following endeavours were undertaken on the initiative of Tomasz Jagielski, Chair of the Organizing Committee of the Pasteur Jubilee Conference.

Louis Pasteur Postage Stamp

We are pleased to announce that a definitive stamp featuring Louis Pasteur, on the occasion of his bicentennial, was issued by the Polish Post.

The issue consists of a stamp (design by Jan Konarzewski), an FDC envelope with a microphotograph of bacteria (photo by Tomasz Jagielski), while the dated postmark features a schematic drawing of a microscope.

The official presentation of the stamp will take place during the Pasteur Jubilee Conference in Warsaw.



Louis Pasteur postage stamp, Poland, 2022.

Louis Pasteur Memorial Medal

On the occasion of the 200th anniversary of the birth of Louis Pasteur, a commemorative medal will be issued by the Mint of Poland. The Medal was designed and sculpted by Anna B. Wątróbska-Wdowiarska, a widely recognized Polish sculptress and medallist.
The medal will be exhibited for the first time at the Pasteur Jubilee Conference in Warsaw.
Details on how to purchase the medal will be provided at the registration desk.

Louis Pasteur, 2022, plaster medal casts.

Anna B. Wątróbska-Wdowiarska studied at the Academy of Fine Arts in Warsaw, receiving her diploma in medallic art in the studio of Zofia Demkowska. Her works have been shown at numerous exhibitions, both home and abroad, and have brought her several awards and honourable mentions. She is the author of many Polish coins issued by the National Bank of Poland.
https://medale-annawatrobska.com/

Louis Pasteur Memorial Medallion

For the celebration of the bicentenary of the birth of Louis Pasteur, a unique ceramic clay medallion, in medium relief, was created by Maryna Szöllősi.

Louis Pasteur, 2022, ceramic clay medallion.

Maryna Szöllősi has a PhD in biology. She worked at the Faculty of Biology, University of Warsaw, until 1985. From 1985 to 1997, she worked at the Institute National de la Recherche Agronomique in France. For many years, she has been developing her artistic passions by sculpting, mostly in clay, and painting in pastel and oil. In France, she worked in the studio of Bernard Grassias (Les Ateliers de la Cour Roland). Her paintings and sculptures have been exhibited at numerous exhibitions. Many of her works are in private collections in Poland and abroad.
https://marynaszollosi.wex.pl/

Acknowledgments

The Organizing Committe is profoundly grateful to Professor Alojzy Nowak, Rector of the University of Warsaw for his interest and support for the project of the Pasteur Jubilee Conference, from its early stages onwards, and for his consent to have the University of Warsaw host this meaningful event.

The Organizing Committee extends its gratitude to the rectors and vice-rectors of universities, directors of research institutes, and heads of other scientific-educational organizations that appreciated the importance of the initiative and supported it, either financially or by a host of other measures.

Great appreciation is expressed to the Ambassador of France in Poland, Frédéric Billet, for the patronage and generous support of the conference. Special thanks are conveyed to Georges Diener, Counsellor for Cooperation and Cultural Action, and Jean-Luc Schneider, Attaché for Scientific and Academic Cooperation, who showed much interest and commitment to the project and its execution.

The Committee wishes to express its most sincere thanks to the following institutions for taking honorary patronage over the conference:

- Marshal of the Senate of the Republic of Poland
- Marshal of the Masovian Voivodeship
- Major of the City of Warsaw

The Committee is grateful to all sponsors and exhibitors for their valuable contribution to the conference.

The following institutions (and their employees) are gratefully acknowledged for their support and contributions to the conference and publication of the conference book:

- Institut Pasteur – President's Office (Louis Marty)

- Institut Pasteur – Scientific Information Resources Center (Marie Martin)
- The Lister Institute of Preventive Medicine (Dina Almuli, Nicola King)
- European Society of Clinical Microbiology and Infectious Diseases (Piotr Kardas)
- Federation of European Microbiological Societies (Joseph Brooks Shuttleworth)
- International Society for Human and Animal Mycology Office (Jacques F. Meis)
- City Hall of Warsaw (Magdalena Olczak)
- Royal Castle in Warsaw – Museum (Lesław Krzewski)
- The Museum of the University of Warsaw (Hubert Kowalski)
- The Museum of Warsaw (Aleksandra Sołtan-Lipska, Kamila Utrata)
- The Warsaw Rising Museum (Barbara Augustyniak-Papież, Martyna Niziurska-Olszaniec)
- Warsaw Tourist Office (Bartosz Milczarczyk, Magdalena Orzełowska)
- Postępy Mikrobiologii [Advancements of Microbiology] Editorial Office (Jacek Bielecki, Radosław Stachowiak, Karolina Jaworska)
- The Library of the Faculty of Biology, University of Warsaw (Izabela Wyszomirska)
- The Mint of Poland (Patrycja Rosłoniec)
- Poczta Polska [Polish Post] (Agnieszka Dyczkowska)

A word of appreciation is due to the Conference Secretariat, and especially Ms. Magdalena Kędzierska for her professional and always timely assistance.

The Organizing Committe thanks the editors of Wydawnictwo Uniwersytetu Warszawskiego, and Małgorzata Yamazaki, in particular, for her outstanding work on the conference book.

The Organizing Committee is also indebted to many individuals who, through their ideas, leverage, and/or actions, made this conference possible, in particular Agata Budkowska, Waleria Hryniewicz, Mateusz Iskra, Henryk Krukowski, Anna B. Macura, Anna Macura-Biegun, Nicolas Maslowski, Maryna Szöllősi, Grzegorz Węgrzyn, Elżbieta Wieteska, Katarzyna Zaborowska and Sławomir Zagórski.

Index of Speakers and Authors

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1. https://szczepieniaprzeciwgrypie.pl/grypa (last access: September 2022)
2. https://www.nhsggc.org.uk/media/248066/6016-a-guide-to-childhood-immunisations-up-to-five-years-of-age-march2018-polish.pdf (last access: September 2022)
3. https://www.health.gov.il/English/Topics/Pregnancy/during/Pages/Vaccination-Whooping_cough.aspx. (last access: September 2022)
4. https://cmsolimed.pl/wp-content/uploads/2020/12/poradnik-podroznika.pdf (last access: September 2022)

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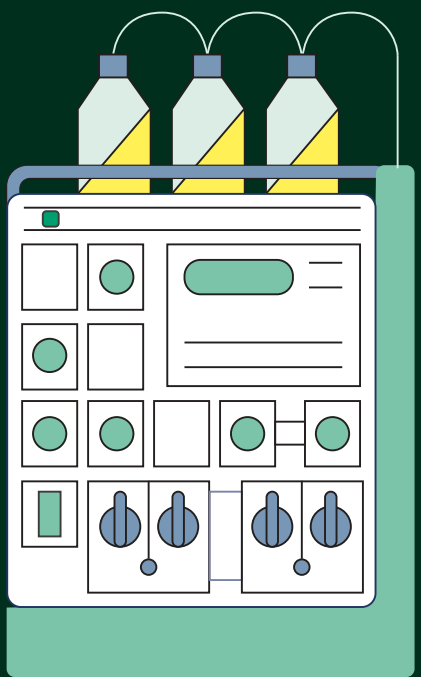

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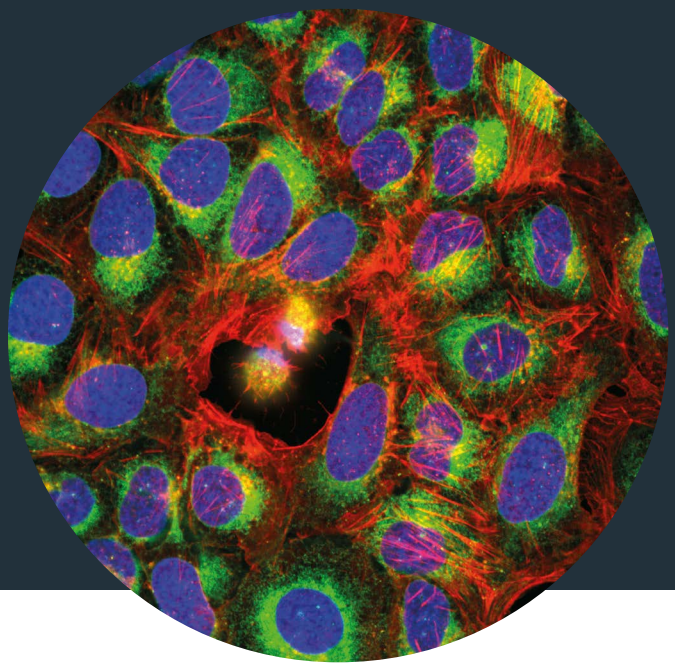

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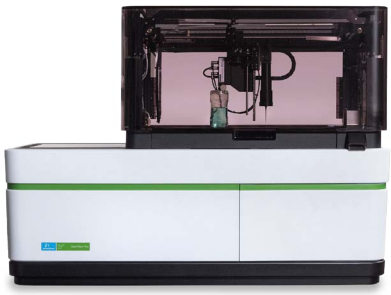

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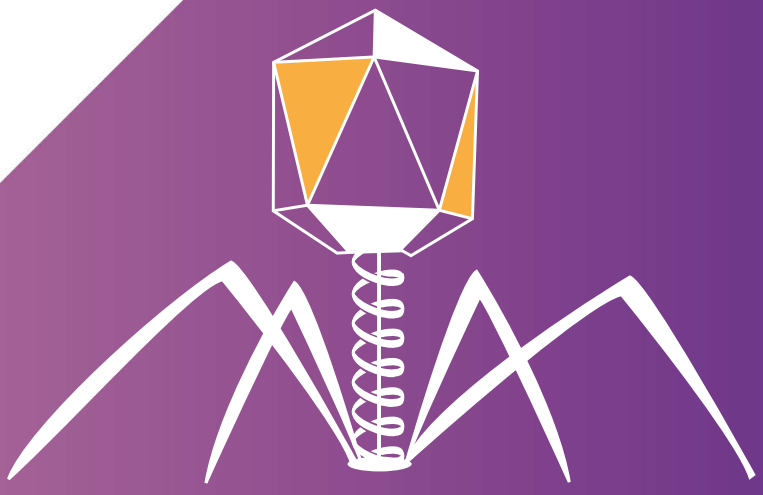

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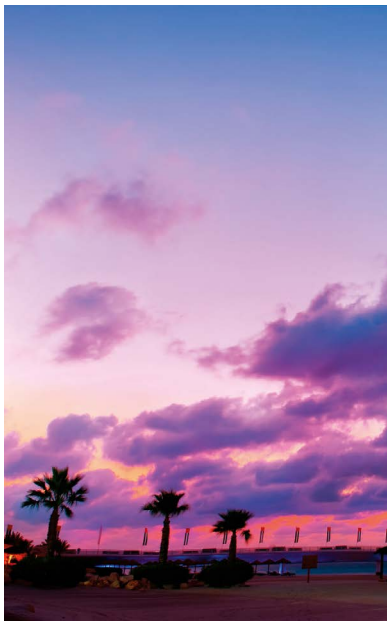

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Layout and Cover Design
Katarzyna A. Jarnuszkiewicz

Cover Illustration
Based on the photograph of Louis Pasteur by Paul Nadar (1886)

Typesetting
ALINEA

Published with the financial support of the Marshal Office of Masovia, the Rector of the University of Warsaw, and University of Warsaw within the Excellence Initiative: Research University (IDUB) programme (no. BOB-IDUB-622-297/2022).

ISBN 978-83-235-5881-1

University of Warsaw Press
PL 02-678 Warsaw, 5/7 Smyczkowa St.
e-mail: wuw@uw.edu.pl
www.wuw.pl

Print and binding
Totem.com.pl

The responsibility for the scientific content of the abstracts is fully borne by the authors.

All additional texts were prepared by the editorial team as listed above.

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