XV Congress of Microbiologists in Bulgaria with International Participation

PROGRAM AND ABSTRACTS

Koprivshtitsa, Bulgaria October 5th – 8th, 2022

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Dedicated to the 200th Anniversary of the birth of Louis Pasteur

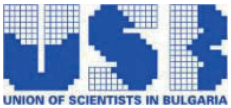
and

75th Anniversary of the Stephan Angeloff Institute of Microbiology at the Bulgarian Academy of Sciences

Koprivshtitsa, October 5th – 8th, 2022

Congress
is organized by

Bulgarian Society for Microbiology



Union of Scientists in Bulgaria

The Stephan Angeloff Institute of Microbiology

Bulgarian Academy of Sciences

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Congress Sections

Covid-19
General and Applied Microbiology (GAM)
Medical Microbiology (MM)
Veterinary Microbiology (VM)
Environmental Microbiology (EM)
Virology (V)
Food Microbiology (FM)
Infectious Immunology (II)

General Program

Wednesday, October 5th, 2022

Transport Sofia – Koprivshtitsa
10:00 – 15:00

Registration

Onset 14:00

18:30 - Opening ceremony

19:00 - Inaugural lecture

20:00 - Welcome Reception

Thursday, October 6th, 2022

9:00 – 18:00 Congress sessions

18:00 – 19:00 Poster session

Friday, October 7th, 2022

9:00 – 18:00 Congress sessions

18:00 – 19:00 Poster session

Saturday, October 8th, 2022

8:30 – 13:00 Congress sessions

13:00 - Congress Adjourn

Social Events

Wednesday – Friday, October 5th – 8th, 2022

Art Exhibitions

Thursday, October 6th, 2022

Tour of the historical town Koprivshtitsa

Friday, October 7th, 2022

20:00 Congress Gala Dinner

PROGRAM

Wednesday, October 5th, 2022
Old School

14:00 **Registration**

18:00 **Congress Opening ceremony**

Angel Galabov, President of the BSM – Congress Opening speech

Congratulatory Addresses

18:30 **Inaugural lecture**

The development of molecular tools for the optimization of *Bacillus* protein secretion

Colin Harwood

Centre for Bacterial Cell Biology, Newcastle University, UK; Treasurer of FEMS

Thursday, October 6th, 2022
Old School

9:30-10:00 **Two Hundred Years Anniversary of Louis Pasteur**
Hristo Najdenski
The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia

Session One (10:00 – 15:00) [Coffee break 11:00 – 11:30]

COVID 19

Chairs: Iva Christova, Athanasios Tsakris

Plenary lectures

10:00-10:30 **Covid1** **Drug resistant septicemic *Escherichia coli* – impact in hospital-acquired infections and during the COVID pandemic**
Eliora Ron
President of IUMS, General Secretary of EAM, Tel-Aviv University, Tel-Aviv, Israel

10:30-11:00 **Covid2** **Molecular-epidemiological, virological and immunological studies on SARS-CoV-2 in Bulgaria (2020-2022)**
Iva Christova, Ivan Ivanov, Ivaylo Alexiev, Nelly Korsun, Maria Nikolova
National Center of Infectious and Parasitic Diseases, Sofia

Coffee break 11:00 – 11:30

11:30-12:00 **Covid3** **Challenges of SARS-CoV-2 diagnostic tests**
A. Arzu Sayner
Department of Medical Microbiology, Medical Faculty, Dokuz Eylul University, Izmir

12:00-12:30 Covid4 SARS CoV 2 virus: rationales of introduction of cocktail antibody

Andi Koraqi, Denada Lacej, Rovena Dhroso
[1]*Department of Microbiology, University of Medicine, Tirana; University Hospital Center "Mother Theresa", Tirana*

12:30-13:00 Covid5 Vaccinations, infections, and reinfections: Can we break the cycle of SARS-CoV-2

Athanasios Tsakris
Department of Microbiology, Medical School, University of Athens, Athens

Lunch break 13:00 -14:00

14:00-14:30 Covid6 Generation and characterization of molecular tools for the study of host immune responses in SARS-CoV-2 infection

Pelagia Foka
Molecular Virology Laboratory, Hellenic Pasteur Institute, Athens

Oral reports

14:30-14:40 Covid7 Accuracy comparison between ELISA and rapid antibody tests in the diagnostics of SARS-CoV-2 infection

Lyubomir Gaydarski[2], Ivo Sirakov[1], Tanja Strateva[1], Dimitar Bakalov[3], Yeshaa Mirani[2], Petja Stankova[1], Nikolay Kalvatchev[1], Raina Gergova[1]
[1]*Department of Medical Microbiology, Faculty of Medicine, Medical University of Sofia;* [2]*Faculty of Medicine, Medical University of Sofi, Sofia;* [3]*Department of Physiology and Pathophysiology, Faculty of Medicine, Medical University of Sofia, Sofia*

Session Two (15:00 – 18:30)]

General and Applied Microbiology

Chairs: Penka Petrova, Colin Harwood

Plenary lectures

15:00-15:30 GAM1 **Profiling the abysso-hadal prokaryotic communities of the Kuril Kamchatka Trench by a metabarcoding approach**

Massimiliano Fenice[1,2], Marcella Pasqualetti[1,3], Susanna Gorrasi[1]
[1]Laboratory of Microbiology, Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy; [2]Laboratory of Applied Marine Microbiology - CoNISMa, Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy; [3]Laboratory of Ecology of Marine Fungi - CoNISMa, Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy

15:30-16:00 GAM2 **Can industrial microbiology provide sustainable alternatives to our slave-like dependence on fossil resources?**

Jean Marie François
Toulouse Biotechnology Institute & Toulouse White Biotechnology Center, INSA-CNRS-INRAe 31077-Toulouse

Coffee break 16:00 – 16:30

16:30-17:00 GAM3 **Physiologically based *in vitro* model to predict the probiotic potential of different lactobacillus strains in combination of natural bioactive substances**

Ilia Iliev, Tonka Vasileva, Veselin Bivolarski, Mariana Nikolova, Daniela Mollova, Ivica Dimov
[1]Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University "Paisii Hilendarski", Plovdiv;[2]Centre of Technologies, Plovdiv University"Paisii Hilendarski", Plovdiv

17:00-17:30 GAM4 **Mechanism of action of antimicrobial peptides from the mucus of the snail *Cornu aspersum***

Pavlina Dolashka[1], Lyudmila Velkova[1], Aleksandar Dolashki[1], Karina Marinova[1], Peter Petrov[1], Ventsseslav Atanasov[1], Dimiter Kaynarov[1], Nevena Ilieva[2], Elena Lilkova[2], Momchil Kermedchiev[3], Mihaela Belouhova[4], Maya Zaharieva[5], Ekaterina Krumova[5], Yana Topalova[4], Hristo Naydenski[5], Maria Angelova[5], Leandar Litov[6], Peicho Petkov[6]

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Oral reports

17:30-17:40 GAM5 **Biodiversity of marine fungi and their potential in biotechnology**

Marcella Pasqualetti[1,2], Massimiliano Fenice[1,3], Martina Braconcini[1] and Susanna Gorrasi[1]

[1]Laboratory of Microbiology, Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy; [2]Laboratory of Ecology of Marine Fungi - CoNISMa, Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy; [3]Laboratory of Applied Marine Microbiology - CoNISMa, Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy

17:40-17:50 GAM6 **Localization in relation to function of sialidases in prokaryotes and eukaryotes**

Roumyana Eneva, Stephan Engibarov, Radoslav Abrashev, Ekaterina Krumova, Yana Gocheva, Vera Kolyovska, Maria Angelova

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia

17:50-18:00 GAM7 **Antibacterial effect of metal nanoparticles synthesized from plant extracts**

Karina Marinova[1], Petar Petrov[1], Valentina Lyubomirova[2], Bogdan Rangelov[3], Stela Atanasova[3], Aleksandar Dolashki[1], Lyudmila Velkova [1], Pavlina Dolashka[1]

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18:00-18:10 GAM8 **Cell response to cold stress in Antarctic fungus *Penicillium gryseofulvum***

Vladislava Dishliyska[1], Galina Stoyancheva[2], Radoslav Abrashev[1], Jeny Miteva-Staleva[1], Boryana Spasova[1], Maria Angelova[1], Ekaterina Krumova[1]

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18:10-18:20 GAM9 **Enzyme profile of lactic acid bacteria strains isolated from breast milk**

Daniela Mollova, Tonka Vasileva, Veselin Bivolarski, Ilia Iliev

Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University "Paisii Hilendarski", Plovdiv

18:20-18:30 GAM10 **New Beta-galactosidases from lactic acid bacteria**

Ivan Ivanov[1], Alexander Arsov[1], Penka Petrova[1], Kaloyan Petrov[2]

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Institute of Chemical Engineering, Bulgarian Academy of Sciences, Sofia

Poster Session One (18:00 – 19:00)

GAM, MM, VM

Chairs: Lyubka Doumanova, Marchella Pasqualeti, Petya Orozova, Radoslav Abrashev

General and Applied Microbiology (GAM)

GAM11 **Cloning and expression of inulinase gene in 2,3-butanediol producing *Bacillus licheniformis* 24**

Lidia Tsigoriyna[1], Alexander Arsov[2], Penka Petrova[2], Kaloyan Petrov[1]
[1]Institute of Chemical Engineering, Bulgarian Academy of Sciences, Sofia; [2]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia

GAM12 **Optimization of the enzyme production of mutant glucosyltransferase URE 13-300 in recombinant strain *Escherichia coli* BL21**

Stanimira Angelova[1], Tonka Vasileva[1], Veselin Bivolarski[1], Ilia Iliev[1,2]
[1]Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University "Paisii Hilendarski", Plovdiv;[2]Centre of Technologies, Plovdiv University"Paisii Hilendarski", Plovdiv

GAM13 **Characterization of glucansucrases producing *Leuconostoc mesenteroides* strains isolated from kefir**

Tonka Vasileva[1], Veselin Bivolarski[1], Iskra Ivanova[3], Penka Moncheva[3], Stanimira Angelova[1], Ilia Iliev[1,2]
[1]Department of Biochemistry and Microbiology, Faculty of Biology Plovdiv University "Paisii Hilendarski", Plovdiv; [2]Centre of Technologies, Plovdiv University, Plovdiv; [3]Department of General and Industrial Microbiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia

GAM14 **Bacteriophage seed coating as prevention approach in combating *Xanthomonas euvesicatoria*-associated plant diseases**

Melani Dimitrova, Yoana Kizheva, Iliyana Rasheva, Petya Hristova
Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia

GAM15 **Sesquiterpene lactones with promising inhibitory effects on bacterial quorum sensing and biofilm formation in gram negative and gram-positive bacteria**

Petya D. Dimitrova[1] Tsvetozara Damyanova[1], Milka Todorova[2], Miroslav Rangelov[2], Nadezhda Todorova[3], Antoaneta Trendafilova[2], Tsvetelina Paunova-Krasteva[1]
[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia; [3]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia

GAM16 ***Inula britannica*** **L. extracts as natural inhibitors for control of bacterial virulence factors**

Petya D. Dimitrova[1], Viktoria Ivanova[2], Antoaneta Trendafilova[2], Tsvetozara Damyanova[1], Dayana Borisova[1], Tsvetelina Paunova-Krasteva[1]

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia;[2]Institute of Organic chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia

GAM17 **Inhibitory capacity of** ***Lactobacillus*** **postbiotics on biofilm formation and quorum sensing activity**

Petya Dimitrova, Lili Dobreva, Tsvetozara Damyanova, Svetla Danova, Tsvetelina Paunova-Krasteva

Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Bulgaria, a member of the International Network of the Institutes Pasteur

GAM18 **Mixed polymeric micelles loaded with ciprofloxacin for the treatment of** ***Staphylococcus aureus*** **biofilm infections**

Tsvetozara Damyanova[1], Tsvetelina Paunova-Krasteva[1], Milena Leseva[1] Rumena Stancheva[2], Petya D. Dimitrova[1], Emi Haladjova[2], Petya A. Dimitrova[1], Stoyanka Stoitsova[1]

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Institute of Polymers, Bulgarian Academy of Sciences, Sofia

GAM19 **Cellular effects on** ***Saccharomyces cerevisiae*** **of chitosan-ferric oxide nanocomposites doped with** Cu^{2+}

Tsvetozara Damyanova*[1], Tsvetelina Paunova-Krasteva[1], Amany M. El Nahrawy[2], Petya D. Dimitrova[1], Mohamed Azab El-Liethy[4], Gamila E. El-Taweel[4], Bahaa A. Hemdan[4], Stoyanka Stoitsova[1]

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Solid-State Physics Department, National Research Centre, 33 El-Bohouth St., Dokki, Giza 12622, Egypt; [3]Water Pollution Research Department, National Research Centre, 33 El-Bohouth St., Dokki, Giza 12622, Egypt

GAM20 **Candidate-probiotic strains from traditional Bulgarian product "Katak"**

Lili Dobreva[1], Petya Dimitrova[1], Tsvetozara Damyanova[1], Tsvetelina Paunova-Krasteva[1] Miglena Koprinarova[2], Vanya Velkova[1], Svetla Danova[1]

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]The Institute of Molecular Biology „Acad. Roumen Tsanev", Bulgarian Academy of Sciences, Sofia

GAM21 **Plant-associated lactic acid bacteria – potential for application**

Tsvetanka Teneva-Angelova

Laboratory of Cell Biosystems Department of Biotechnology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Plovdiv

GAM22 **Effect of lysates from plant-associated bacteria on secondary metabolite profiles of** ***Salvia ringens*** **adventitious roots culture**

Tsvetanka Teneva-Angelova, Vasil Georgiev

Laboratory of Cell Biosystems Department of Biotechnology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Plovdiv

GAM23 **Isolation and identification of lactic acid bacteria from infant faeces**

Sandra Stanimirov[1], Melani Dimitrova[1], Maria Pandova[1], Radoslav Rangelov[2], Yoana Kizheva[1], Petya Hristova[1], Iliyana Rasheva[1*]

[1]Department of General and Industrial Microbiology, Biological Faculty, Sofia University "St. Kliment Ohridski"; [2]Vita Hospital,Sofia

GAM24 **Diversity of the cloacal aerobic bacterial flora of free-living lizards from Ihtimanska Sredna Gora**

Irina Lazarkevich[1], Stefan Engibarov[1], Simona Mitova[1], Emilyia Vacheva[2], Nickola Stanchev[3], Steliyana Popova[3]

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences; [2]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences; [3]Faculty of Biology, Sofia University „St Kliment Ohridski"

GAM25 **Chemical composition and antimicrobial activity of essential oils from Bulgarian *Epilobium parviflorum* Schreb**

Tzenka Radoukova[1], Yovko Dyulgerski[2], Ivelina Zapryanova[3]

[1]Department of Botany and Biological education, Faculty of Biology, University of Plovdiv "Paisii Hilendarski", Plovdiv; [2]Tobacco and Tobacco Products Institute, Markovo, Bulgaria; [3]Department of Animal science, Agricultural Faculty, Agricultural University, Plovdiv

Medical Microbiology (MM)

MM12 **Antimicrobial effect of siloxane coatings with antioxidants**

Desislava Petkova Boteva[1], Kalina Emil Ivanova[1], Todorka G. Vladkova[3], Iliana Atanassova Ivanova[2]

[1]Bachelor degree in Molecular Biology 2022, Sofia University "St. Kliment Ohridski"; [2]Departament General and Industrial Microbiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia; [3] University of Chemical Technology and Metallurgy, Sofia

MM13 **PCR analysis for *Mycobacterium tuberculosis* complex shows negative results in sarcoid samples**

B. Tsafarova[1], Y. Hodzhev[1], V. Tolchkov[1], V. Youroucova[2], S. Ivanova[2], D. Kostadinov[2], N. Yanev[2], S. Panaiotov[1]

[1]National Center of Infectious and Parasitic Diseases, Sofia; [2]Department of Pulmonary Disease,s Medical Faculty, Medical University of Sofia, Sofia

MM14 **Antimicrobial effect of magnetic nanoceramics**

Kalina Emil Ivanova[1], Desislava Petkova Boteva[1], Iliana Atanassova Ivanova[2], Peter Georgiev[3]

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MM15 **Effects of autochthonous (wild) *Lactocaseibacillus paracasei* strains on ALP activity in non-differentiated and differentiated human colorectal adenocarcinoma HT 29 cells**

Valeria Petrova[1], Natalia Grigorova[1], Zhenya Ivanova[1], Daniela Stoeva[3], Georgi Beev[2], Ekaterina Vachkova[1]

[1]Department of Pharmacology, Animal Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, Stara Zagora; [2]Department of Biochemistry, Microbiology and Physicsmicrobiology, Faculty of Agriculture, Trakia University, Stara Zagora; [3]Central Scientific and Research Laboratory, Trakia University, Stara Zagora

MM16 **Autochthonous *Lactocaseibacillus paracasei* strains enhance glucose uptake in mature 3T3-L1 adipocytes**

Zhenya Ivanova[1], Ekaterina Vachkova[1], Valeria Petrova[1], Daniela Stoeva[3], Georgi Beev[2], Natalia Grigorova[1]

[1]Department of Pharmacology, Animal Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, Stara Zagora; [2]Department of Biochemistry, Microbiology and Physicsmicrobiology, Faculty of Agriculture, Trakia University, Stara Zagora; [3]Central Scientific and Research Laboratory, Trakia University, Stara Zagora

Veterinary Microbiology (VM)

VM7 **Therapeutic effect of electrochemically activated aqueous solutions for the treatment of infections of various origins in dogs**

Toshka Petrova[1*], Milko Petrov[2], Mila Kaleva[3,4,*] , Teodora Popova[1], Ignat Ignatov[5], Hristo Najdenski[3]

**These authors contributed equally*

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VM8 **Detection of some genes encoding antibiotic resistance among *Staphylococcus aureus* isolates from cows with mastitis in Bulgaria**

Nikolina Velizarova Rusenova[1*], Nasko Yovchev Vasilev[2], Anton Georgiev Rusenov[3], Aneliya Milanova Milanova[4], Ivo Nikolaev Sirakov[5]

[1]Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora; [2]Department of Obstetrics, Reproduction and Reproductive Disorders, Faculty of Veterinary Medicine, Trakia University, Stara Zagora; [3]Department of Internal Noninfectious Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora; [4]Department of Pharmacology, Animal Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, Stara Zagora; [5]Department of Medical Microbiology, Faculty of Medicine, Medical University of Sofia, Sofia

VM9 **Pilot study on the representatives of genus *Vibrio* in the Bulgarian Black Sea area**

Petya Orozova, Roumyana Nenova, Iskra Tomova, Ognyana Hristova, Lyudmila Gorjanova, Dimitar Ivanov

[1]National Reference Laboratory for Fish, Mollusk and Crustacean Diseases, National Diagnostic and Research Veterinary Medical Institute, Sofia; [2]National Reference Laboratory Special Bacterial Pathogens National Center of Infectious and Parasitic Diseases, Sofia; [3]Institute of Oceanology, Bulgarian Academy of Sciences, Varna

VM10 **Changes in the activity of aspartate-alanine aminotransferase in dogs with experimentally induced *Staphylococcus aureus* infection**

Dimitrinka Zapryanova[1], Nikolina Rusenova[2], Georgi Beev[3], Parvan Parvanov[2], Teodora Mircheva[1], Stefan Denev[2], Deyan Georgiev[4]

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VM11 ***In vitro* antibacterial effect of *Lemna minuta*, *Chlorella vulgaris*, and *Spirulina* sp. extracts against fish pathogen *Aeromonas hydrophila***

Katya Velichkova[1], Ivajlo Sirakov[1], Stefan Denev[2]

[1]Department of Biology and Aquaculture, Faculty of Agriculture, Trakia University, Stara Zagora; [2]Department of Biochemistry, Microbiology and Physics, Faculty of Agriculture, Trakia University, Stara Zagora

Friday October 7th, 2022

Old School

Session Three (9:00 – 13:00) [Coffee break 11:00 – 11:30]

Medical Microbiology

Chairs: Marianna Murdjeva, Igor Mokrousov

Plenary lectures

9:00-9:30 MM1 **Epidemic and endemic *Mycobacterium tuberculosis* clusters in Russia: pathobiology and phytogeography**
Igor Mokrousov
St. Petersburg Pasteur Institute, St. Petersburg

9:30-10:00 MM2 **Multiplex PCR for modern syndromic microbiological diagnosis of severe infections**
Marianna Murdjeva[1,2,3], Yordan Kalchev [1,2,3], Gergana Lengerova[1,2,3], Andreana Angelova[1,2,3]
[1]Department of Medical Microbiology and Immunology „Prof. Dr. Elissay Yanev", Faculty of Pharmacy, Medical University, Plovdiv; [2]Laboratory of Microbiology, University Hospital St. George, Plovdiv; [3]Research Institute at Medical University, Plovdiv

10:00-10:30 MM3 **Historical review of tuberculosis vaccines in Bulgaria: complete genome sequence, genome stability and phylogeny of the vaccine strain *Mycobacterium bovis* BCG SL222 Sofia**
Stefan Panaiotov[1], Yordan Hodzhev[1], Vladimir Tolchkov[1], Borislava Tsafarova[1], Alexander Mihailov[2], and Tzvetelina Stefanova[2]
[1]National Center of Infectious and Parasitic Diseases, Sofia; [2]BB-NCIPD Ltd., Sofia, Bulgaria

10:30-11:00 MM4 **Antimicrobial stewardships – parallels**
Emma Keuleyan
University Multi-Profile Hospital "Lozenetz", Sofia

Coffee break 11:00 – 11:30

11:30-12:00 MM5 **Targeting glmS and FMN riboswitches with antisense oligonucleotides for antibacterial drug development**

Robert Penchovsky, Martina Traykovska
Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia

Oral reports

12:00-12:10 MM6 **Genomic characterization of unusual case of facial nec rotizing fasciitis, caused by a novel methicilin-sensitive *Staphylococcus aureus* sequence type (cc398-t1451)**

Ivan N. Ivanov[1], Denis Niyazi[2], Ivan Stoykov[1], Milena Bozhkova[2], Borislava Toncheva[3], Tsvetan Tonchev[3], Deyan Donchev[1], Stefan L ozenov[1], Elina Dobreva[1], Rumyana Hristova[1], Temenuga Stoeva[2]
[1]National Reference Laboratory for Control and Monitoring of Antimicrobial Res istance, National Center of Infectious and Parasitic Diseases, Sofia; [2]Laboratory of Clinical Microbiology, University Hospital "St. Marina", Varna; [3]Maxillofaci al Surgery Clinic, University Hospital "St. Marina", Varna

12:10-12:20 MM7 **Characterization of cefiderocol resistance in multi-drug resistant *Pseudomonas aeruginosa* by whole genome sequencing – preliminary results**

Ivan N. Ivanov[1], Ivan Stoikov[1], Deyan Donchev[1], Elina Dobreva[1], Rumyana Hristova[1], Stefan Lozenov[1], Stefana Sabtcheva[2]
[1]National Reference Laboratory for Control and Monitoring of Antimicrobial Resistance, National Center of Infectious and Parasitic Diseases, Sofia; [2]National Oncology Center, Sofia

12:20-12:30 MM8 ***Clostridium difficile* infection among COVID-19**

Maria Pavlova[1], Ekaterina Alexandrova[1], Ivan Ivanov[2], Andreana A ngelova[3], Eli Hristozova[3], Jordan Kalchev[3], Ivan Lyutakov[4], Valeri Velev[2]
[1]Department of Microbiology, National Centre of Infectious and Parasitic Diseases, Sofia; [2]University Hospital "Prof. Iv. Kirov", Medical University, Sofia; [3]Department of Microbiology and Immunology, Medical University, Plovdiv, University Hospital "St. Georgi", Plovdiv; [4]University Hospital "Queen Joanna - ISUL," Medical University, Sofia

12:30-12:40 MM9 **Novel composites based on activated carbon and Cu, Ag, and Mg as antibacterial agents**

Marieyta Belcheva[1], Georgi Georgiev [2], Venelin Hubenov[1], I. Stoycheva[2], B. Tsyntsarski[2], Lyudmila Kabaivanova[1]
[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, [2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia

12:40-12:50 MM10 Primary multidrug resistance and major clusters of the epidemiologically significant *Mycobacterium tuberculosis* Beijing genotype in Northwest Russia

Anna Vyazovaya[1], Alena Gerasimova[1], Natalia Solovieva[2], Viacheslav Zhuravlev[2], Igor Mokrousov[1]

[1]St. Petersburg Pasteur Institute, St. Petersburg, Russia; [2]St. Petersburg Research Institute of Phthisiopulmonology, St. Petersburg, Russia

12:50-13:00 MM11 Direct identification of *Yersinia pseudotuberculosis* in raw milk by digital droplet PCR and loop mediated isothermal amplification

Maya Zaharieva[1], Tanya Chan Kim[1], Mila Kaleva[1], Lyudmila Dimitrova[1], Yana Ilieva[1], Els van Collie[2], Mark Heyndrickx[2], Hristo Najdenski[1]

[1]Department of Infectious Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Unit "Food Safety", Institute for Agriculture and Fisheries Research (ILVO), Melle, Belgium

Lunch break 13:00 -14:00

Session Four (14:00 – 18:00) [Coffee break 16:10 – 16:30]

VETERINARY MICROBIOLOGY

Chairs: Hristo Najdenski, Raiko Peshev

Plenary lectures

14:00-14:30 VM1 Role of pig farms in the spreading of antimicrobial resistance

Lyudmila Dimitrova, Maya M. Zaharieva, Mila D. Kaleva, Yana Ilieva, Maya Angelovska, Vesselin Kussovski, Hristo Najdenski

The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia

14:30-15:00 VM2 Studies on herpesvirus infections in ruminants

Raiko Peshev

National Diagnostic and Research Veterinary Medical Institute, "Prof. Dr. G. Pavlov" Sofia

Oral reports

15:00-15:10 VM3

Isolation of *Flavobacterium psychrophilum* from cultured rainbow trout (*Oncorhynchus mykiss*) in Bulgaria

Petya Orozova[1], Dimitar Ivanov[1], Ivan Stoykov[2], Deyan Donchev[2], Ivan Ivanov[2]

[1]National Reference Laboratory for Fish, Mollusk and Crustacean Diseases, National Diagnostic and Research Veterinary Medical Institute, Sofia; [2]National Reference Laboratory for Control and Monitoring of Antimicrobial Resistance, National Center of Infectious and Parasitic Diseases, Sofia

15:10-15:20 VM4

First isolation and identification of *V. aestuarianus* from the Bulgarian sea area

Petya Orozova[1], Rumyana Nenova[2], Iskra Tomova[2], Ognyana Hristova, Dimitar Ivanov[1]

[1]National Reference Laboratory for Fish, Mollusk and Crustacean Diseases, National Diagnostic and Research Veterinary Medical Institute, Sofia; [2]National Reference Laboratory Special Bacterial Pathogens National Center of Infectious and Parasitic Diseases, Sofia; [3]Institute of Oceanology, Bulgarian Academy of Sciences, Varna

15:20-15:30 VM5

Microbiological studies on the prevalence of *Staphylococcus* spp., involved in the etiology of mastitis in cattle and their susceptibility to antimicrobial agents in the Republic of Bulgaria

Mariana Nikolova, Valentina Urumova

Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora

15:30-15:40 VM6

On some basic characteristics of *Streptococcus suis* strain isolated from intensive reared pigs in Bulgaria

Vasilena Galeva

Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Veterinary Faculty at the Trakia University, Stara Zagora

ENVIRONMENTAL MICROBIOLOGY

Chairs: Ekaterina Krumova, Massimiliano Fenice

Plenary lectures

15:30-16:00 EM1 **The control of the Valuable Biological Materials in an unsafe world**

Gabriel Ionescu
„Cantacuzino" National Military Medical Institute for Research and Development; „Carol Davila" University of Medicine and Pharmacy Bucharest

Coffee break 16:00 – 16:30

16:30-17:00 EM2 **Two-stage anaerobic digestion of organic wastes: state of the art and some perspectives**

Ivan Simeonov, Lyudmila Kabaivanova, Elena Chorukova
The Stephan Angeloff Institute of Microbiuology, Bulgarian Academy of Sciences, Sofia

17:00-17:30 EM3 **Multifunctional algology lab for maximum CO_2 capture and synthesis of high value products. Modern challenges of climate changes and virus pandemic**

Alexander Dimitrov Kroumov[1], Maya Margaritova Zaharieva[1], Yana Illieva[1], Mila Kaleva[1], Tanya Kim[1], Fabiano Bisinella Scheufele[2], Daniela Estelita Goes Trigueros[2], Aparecido Nivaldo Módenes[2], Hristo Najdenski[1]
[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Department of Chemical Engineering, Nucleus of Biotechnology Center, West Paraná State University-Toledo, Toledo, PR, Brazil

Oral reports

17:30-17.40 EM4 **Metabarcoding characterisation of the bacterial diversity within the thalassohaline environment of the saline di Tarquinia, Italy**

Susanna Gorrasi[1], Marcella Pasqualetti[1,2], Massimiliano Fenice[1,3]
[1]Laboratory of Microbiology, Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy; [2]Laboratory of Ecology of Marine Fungi, CoNISMa, Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy; [3]Laboratory of Applied Marine Microbiology, CoNISMa, Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy

17:40-17:50 EM5 **Composition and antibacterial effect of water from mineral springs in the region of Velingrad**

Peter Petrov[1], Karina Marinova[1], Valentina Lyubomirova[2], Narzislav Petrov[1], Lyudmila Velkova[1], Angelina Kosateva[1], Pavlina Dolashka[1]

[1]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia; [2]Faculty of Chemistry and Pharmacy of Sofia University "St. Kliment Ohridski", Sofia

17:50-18:00 EM6 **Microbiological, physicochemical and isotopic results of glacier water from Chile**

Ignat Ignatov[1], Nedyalka Valcheva[2]

[1]Scientific Research Center of Medical Biophysics, Sofia; [2]Faculty of Agriculture, Department Biochemistry, Microbiology, Physics, Trakia University, Stara Zagora

Poster Session Two (18:00 – 19:00)

FM, V, EM

Chairs: Lyubka Doumanova, Marchella Pasqualeti, Petya Orozova, Radoslav Abrashev

Virology (V)

V7 **Real time quantitative PCR as a reliable method for detecting CMV reactivation in patients with lymphomas**

Zhivka Stoykova[1,2], Tsvetelina Kostadinova[1,3], Ilina Micheva[4], Tatina Todorova[1,2]
[1]Laboratory of Virology, University Hospital "St. Marina", Varna; [2]Department of Microbiology and Virology, Medical University of Varna, Varna; [3]Medical College, Medical University of Varna, Varna; [4]Clinic of Hematology, University Hospital" St. Marina", Varna

V8 **Combined antiviral treatment against Coxsackievirus B3 and Poliovirus**

Adelina Stoyanova[1], Simeon Galabov[1], Philipov S.[2], Makarov V.[3], Angel S. Galabov[1]
[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia; [3]Bach Institute of Biochemistry, Moscow, Russian Federation

V9 **Treatment *via* consecutive alternating administration of antivirals against Coxsackievirus B3 infection in mice**

Adelina Stoyanova[1], Nikoleta Hristova[1], Simeon Galabov[1], Makarov V.[2], Philipov S.[3], Pürstinger G.[4], Dobrikov G.[3], Angel S. Galabov[1]
[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Bach Institute of Biochemistry, Moscow, Russian Federation; [3]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia; [4]Institute of Pharmacy, University of Innsbruck, Innsbruck, Austria

V10 **Effect of castalagin against HSV-1 infection in mice**

Adelina Stoyanova[1], Victor Rashev [1], Popatanasov A.[2], Lyubka Tancheva[2], Quideau S.[3], Angel S. Galabov[1]
[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia; [3]University of Bordeaux, Bordeaux, France

V11 **SARS-CoV2 spike, enveloped and membrane proteins expressed in *Nicotiana benthamina* detect specific antibodies in COVID-19 patients**

Katerina Takova[1], Jae-Wang Jung[2], George Lomonossoff[2], Gergana Zahmanova[1,3]
[1]Department of Plant Physiology and Molecular Biology, University of Plovdiv, Plovdiv;
[2]Department of Biochemistry and Metabolism, John Innes Centre, Norwich Research Park, UK;
[3]Center of Plant Systems Biology and Biotechnology, Plovdiv

V12 **Data on the prevalence of hepatitis E virus among domestic pigs and wild boar in bulgaria**

Katerina Takova[1], Gergana Zahmanova[1, 2]
[1]Department of Plant Physiology and Molecular Biology, University of Plovdiv, Plovdiv; [2]Center of Plant Systems Biology and Biotechnology, Plovdiv

V13 **Elevation of liver enzymes during Covid-19 pandemics – Bulgarian study on 3038 patients**

Sonja Velkova[1,2], Kalin Hinov[2], Galina Satchanska[1]
[1]Department of Natural Sciences, New Bulgarian University, Sofia; [2]Nadejda-1 Clinical Laboratory, Sofia

Food Microbiology (FM)

FM5 **Effects of *Lactobacillus plantarum* post-metabolites on HT-29 intestinal epithelial cell monolayer characteristics**

Kirilka Mladenova[1], Ralitsa Veleva[1], Lili Dobreva[2], Svetla Danova[2], Pavel Videv[1], Tanya Topouzova-Hristova[1], Denitza Melnishka[1], Jordan Doumanov [1], Vesselina Moskova-Doumanova[1]
[1]Faculty of Biology, Sofia University St. Kliment Ohridski, Sofia [2] The Stephan Angeloff Institut of Microbiology, Bulgarian Academy of Sciences, Sofia

FM6 **Proteolytic activity of different lactic acid bacteria species cultivated on media with plant proteins**

Teodora Panayotova[1,2], Zoltan Urshev[2], Ilia Iliev
[1]Centre of Technologies, Plovdiv University "Paisii Hilendarski", Plovdiv; [2]LB-Bulgaricum Plc., R&D Center, Sofia

FM7 **Cultivation of probiotic lactic acid bacteria species with different proteolytic activity into media based on plant proteins**

Nora Pavlova, Teodora Panayotova, K. Pashova
LB-Bulgaricum Plc., R&D Center, Sofia

FM8 **Virulence potential of enterococci isolates from non-hospital origin**

Maria Pandova, Yoana Kizheva, Iliyana Rasheva, Petia Hristova
Department of General and Applied Microbiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia

FM9 **Antibiotic resistance of enterococci with different origin**

Maria Pandova, Yoana Kizheva, Iliyana Rasheva, Petia Hristova
Department of General and Applied Microbiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia

FM10 **Assessment of the ability of *Streptococcus thermophilus* strains to produce folates**

Irina Gotova, Zhechko Dimitrov
LB Bulgaricum Plc, R&D Center, Sofia

Environmental Microbiology (EM)

EM7 **Influence of urbanization along the Yantra river on the prevalence of antibiotic resistant bacteria**

Zvezdimira Tsvetanova, Hristo Najdenski

Department of Infectious Microbiology, The Stephan Angeloff Institute of Microbiology, Sofia

EM8 **Antimicrobial resistance of drinking water and drinking water-associated biofilms**

Zvezdimira Tsvetanova, Iva Tsvetkova, Hristo Najdenski

Department of Infectious Microbiology, The Stephan Angeloff Institute of Microbiology, Sofia

EM9 **Scientific project "Caves as a reservoir for novel and reoccurring zoonoses - ecological monitoring and metagenomic analysis in real time"**

Vladimir Tolchkov[1], Yordan Hodzhev[1], Borislava Tsafarova[1], Rumyana Nenova[1], Ognyan Mikov[1], Nikolay Simov[2], Gancho Slavov[2], Staninmira Deleva[2], Nia Toshkova[2], Nedyalko Nedyalkov[2], Mario Langurov[2], Rostislav Bekchiev[2], Pavel Stoev[2], Milena Nikolova[2], Stefan Panaiotov[1]

[1]National Center of Infectious Diseases, Sofia; [2]Nationall Museum of Natural History, Bulgarian Academy of Sciences, Sofia

EM10 **Application of bacteriophage-based biopesticide in rhizosphere soil and influence on beneficial soil microorganisms**

Melani Dimitrova, Yoana Kizheva, Iliyana Rasheva, Petya Hristova

Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia

EM11 **Diurnal variations in the concentration of microbial bioaerosoles in outdoor air of highly urbanized Sofia city center**

Ralitsa Ilieva[1], Boyanka Angelova[1], Mihail Iliev[1], Veneta Groudeva[1], Ivan Nedkov[2]

[1]Sofia University "St. Kliment Ohridski", Sofia; [2]Institute of Electronics "Emil Djakov", Bulgarian Academy of Sciences, Sofia

EM12 **Extracellular sialidase enzyme production by filamentous fungi from Livingston Island, Antarctica**

Radoslav Abrashev[1], Ekaterina Krumova[1], Penka Petrova[1], Rumyana Eneva[1], Nedelina Kostadinova[1], Jeni Miteva-Staleva[1], Stephan Engibarov[1], Galina Stoyancheva[1], Yana Gocheva[1], Vera Kolyovska[2], Vladislava Dishliyska[1], Boryana Spassova[1], Maria Angelova[1]

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia

EM13 **Preliminary study of genetic diversity among Rosa genotypes by ISSR markers**

Mariya Zhelyazkova[1], Neli Grozeva[2], Ana Dobreva[3], Veselina Badzhelova[3], Svetlana Georgieva[1]

[1]Department of Genetics, Breeding and Reproduction, Faculty of Agriculture, Trakia University, Stara Zagora; [2]Department of Biology and Aquaculture, Faculty of Agriculture, Trakia University, Stara Zagora; [3]Institute for Roses and Aromatic Plants, Kazanlak, Bulgaria

EM14 **First inside view on microbial community of a graphite mine**

Diliana D. Simeonova, Venelin N. Hubenov

The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia

Saturday, October 8th, 2022

Old School

Session Five (9:00 – 13:00) [Coffee break 11:00 – 11:30]

VIROLOGY

Chairs: Angel Galabov, A. Arzu Sayner

9:00-9:10 **90th Anniversary of the birth of Professor Stephan Dundarov**

Angel Galabov, President of the BSM

The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia

Oral reports

9:10-9:20 **V1** **Correlation between HBSAG quantitative assay results and HBV DNA levels in chronic hepatitis b patients – a single-center experience**

Zhivka Stoykova[1,2], Tsvetelina Kostadinova[1,3], Tatina Todorova[1,2], Irina Ivanova[4,5]

[1]Laboratory of Virology, University Hospital "St. Marina", Varna; [2]Department of Microbiology and Virology, Medical University of Varna, [3]Varna; Medical College, Medical University of Varna, Varna; [4]Clinic of Hepatogastroenterology, University Hospital" St. Marina", Varna; [5]Department of Internal Medicine, Medical University of Varna, Varna

9:20-9:30 V2

Analysis of the transmitted drug resistance and molecular epidemiology of HIV-1 in Bulgaria, challenges, and prospects

Ivaylo Alexiev[1], Alexandra Partsuneva[1], Lyubomira Grigorova[1], Reneta Dimitrova[1], Anna Gancheva[1], Asya Kostadinova[1], Ivaylo Elenkov[2], Nina Yancheva[2], R. Grozdeva[2], Mariana Stoycheva[3], Tsetsa Doichinova[4], Lilia Pekova[5], Minas Kosmidis[6], Radoslava Emilova[7], Maria Nikolova[7]

[1]National Reference Confirmatory Laboratory of HIV, NCIPD, Sofia; [2]Specialized Hospital for Infectious and Parasitic Diseases, Sofia; [3]Department of Infectious Diseases, Medical University, Plovdiv; [4]Department of Infectious Diseases, Medical University, Pleven; [5]Clinic of Infectious Diseases, Medical University, Stara Zagora; [6]Clinic of Infectious Diseases, Medical University, Varna; [7]National Reference Laboratory of Immunology, NCIPD, Sofia

9:30-9:40 V3

Gene silencing of some viral genes of human *Coxsackievirus B*

Nikolay M. Petrov[1], Mariya I. Stoyanova[2], Angel S. Galabov

[1]Laboratory of Virology, Department of Natural Sciences, New Bulgarian University, Sofia; [2]Department of Plant Protection, Institute of Soil Science, Agrotechnologies and Plant Protection "N. Pushkarov", Agricultural Academy, Sofia; The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia

9:40-9:50 V4

Bronchial epithelial cells at the air-liquid-interface for investigation of host-influenza virus interactions

Lora Simeonova[1], Adriana Kaleva[1], Petya Dimitrova[2], Cyril Barbezange[3], Milena Leseva[2]

[1]Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Department of Immunology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [3]Sciensano, National Influenza Centre, Brussels, Belgium

9:50-10:00 V5

Spatio-temporal dynamics of co-circulating rabies virus variants isolated in dog population in Cameroon

Jocelyne Noel Sowe Wobessi[1], Jean Blaise Momo[1], Laura Besong[2], S everin Loul[2], Jean-Luc Bailly[3], Richard Njouom[1], Serge Sadeuh-Mba[1]

[1]Virology Service, Centre Pasteur du Cameroun, Yaounde; [2]Ministry of Livestock, Fisheries and Animal Industries (MINEPIA); [3]Laboratoire Génome Micro-organisme et Environnement (LMGE), Clermont Ferran

10:00-10:10 V6

Removal of viruses from the air by adsorption with activated carbon

Ivanka Stoycheva[1], Boyko Tsyntsarski[1], B. Petrova[1], Angelina Kosateva[1], Narzislav Petrov[1], Stoyan Shishkov[2]

[1]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences Sofia; [2]Laboratory of Virology, Department of Biology, Sofia University "St. Kliment Ohridski", Sofia

FOOD MICROBIOLOGY

Chairs: Svetla Danova, Galina Satchanska

Plenary lectures

10:10-10:40 FM1 **Yeast probiotics in ruminant nutrition and health: a review**

Stefan Denev

Department of Biochemistry and Microbiology, Faculty of Agriculture, Trakia University, Stara Zagora

10:40-11:10 FM2 **Mode of the beneficial effects of Bulgarian probiotic lactobacilli**

Svetla Danova[1], Lili Dobreva[1], Veronika Nemska[1,2], Daiana Borisova[1], Cvetelina Paunova[1], Miglena Koprinarova[3], Jordan Doumanov[4], Tanya Topuzova[4], Veselina Moskova[4]

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]University of Chemical Technology and Metallurgy, Sofia; [3]Institute of Molecular Biology „Acad. Roumen Tsanev", Bulgarian Academy of Sciences, Sofia ;[4]Biological Faculty, Sofia University"St. Kliment Ohridski", Sofia

11:10-11:40 FM3 **Pathogenic bacteria in milk products and their antibiotic resistance**

Galina Satchanska

Department Natural Sciences, New Bulgarian University, Sofia

Oral reports

11:40-11:50 FM4 **Microflora of water samples from Sofia City and Sofia region**

Lilia Petrova Yordanova[1], Iliana Atanassova Ivanova[2], Lyubomira Dimitrova Yocheva[3]

[1]Master degree in Quality and safety of food 2022, Sofia University "St. Kliment Ohridski", Sofia; [2]Department of General and Industrial Microbiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia; [3]Faculty of Medicine, Sofia University "St. Kliment Ohridski", Sofia

INFECTIOUS IMMUNOLOGY

Chairs: Petya Dimitrova, Anastas Pashov

12:00-12:30 **II1** **Targeting neutrophils during inflammation**

Petya Dimitrova, Milena Leseva
Laboratory of Experimental Immunotherapy, Department of Immunology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia

12:30-13:00 **II2** **Global analysis of antibody repertoires**

Anastas Pashov[1], Shina Pashova[2]
[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Insitute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia

13:00 - Congress Adjourn

ABSTRACT BOOK

Inaugural lecture

THE DEVELOPMENT OF MOLECULAR TOOLS FOR THE OPTIMIZATION OF *BACILLUS* PROTEIN SECRETION

Colin Harwood

Centre for Bacterial Cell Biology, Newcastle University, UK

Bacillus subtilis and close relatives are among the most versatile and widely exploited industrial microorganisms. *Bacillus spp.* are used for the production of a variety of commercially important products, including industrial enzymes, vitamins, amino acids, antifungal and antibacterial peptides. Analysis of this bacterium over more than 60 years has revealed detailed knowledge of its biochemistry, physiology and genetics, making it one of the most amenable host bacteria for synthetic biology/industrial biotechnology processes. *B. subtilis* efficiently secretes native proteins and those from related bacteria at concentrations in excess of 20 g/L. However, yields of heterologous proteins, such as therapeutic proteins, are much more variable (µg – mg/L). To better understand the *Bacillus* secretion pathway, we have systematically studied the process from the emergence of a secretory protein from the ribosome to its trafficking to the membrane, wall or culture medium. In so doing, we have identified, and attempted to overcome, various bottlenecks in this pathway.

The main characteristic that limits the secretion of a particular protein is its folding characteristics. *Bacillus* has efficient intracellular and extracellular quality control factors that detect and remove inappropriately folded proteins. This talk will review our current understanding of the *Bacillus* protein secretion pathway and discuss the strains and molecular tools that have been developed to enhance the productivity of this bacterium for a wider range of products.

Session Jubilee Pasteur

TWO HUNDRED YEARS' ANNIVERSARY OF LOUIS PASTEUR

Hristo Najdenski

The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia

Throughout its thousand-year history, humanity has always moved ahead towards improvement, thanks to the pleiad of scientists and scientific discoveries made. Science - this magical activity has not only affected people's imaginations, but has also constantly changed and increased the length and quality of people's lives.

In this year, humanity celebrates the 200th Anniversary of the birth of Louis Pasteur - a titan of science who dedicated his life to microbiology and long-term intense work to reveal the secrets of "those infinitesimal animals called microbes" and their role both in the biotechnological processes and as causative agents of infectious diseases and the related human suffering. Pasteur's activity is extensive and many-sided. It begins with research and discoveries in the field of crystallography, goes through studies on fermentations, refutes the theory of spontaneous generation, sheds light on the most important field of biology - microbiology, and lays the beginning of modern immunobiological studies.

Much has been written about Pasteur and in almost every language, but even today his work continues to develop, and we will once again present our latest achievements in the field of microbiological science. And let's not forget his words "Do not advance anything that cannot be verified simply and decisively. Have a cult of critical spirit. It always has the last word." So, Pasteur's words, the clear logic of his thought, his boundless studiousness and will, his dedication to science are the basis of the huge successes achieved in a short human life.

Humanity respects and will continue to draw inspiration from Pasteur – a genius scientists and benefactors!

COVID-19

Covid1

THE THREAT OF ANTIBIOTIC RESISTANT BACTERIA: BEYOND COVID-19

Eliora Z. Ron

President of IUMS, General Secretary of EAM, Tel-Aviv University, Tel-Aviv, Israel

Corresponding author: eliora@tauex.tau.ac.il

Extraintestinal pathogenic *Esherichia coli* are a severe world-wide problem in hospitals and in the community, especially as they are often resistant to most clinically used antibiotics. We have been studying the healthcare-associated infections caused by these *E. coli* strains and the severe consequences of these infections, especially at the time of the Covid-19 pandemic. During the pandemic there was a significant increase in the use of antibiotics and therefore a selection of resistant bacteria. The Covid-19 pandemic is declining with the availability of vaccines but beyond this pandemic is the growing threat of healthcare/hospital associated infections by antimicrobial resistant bacteria, which so far we have not succeeded to combat.

Covid2

MOLECULAR-EPIDEMIOLOGICAL, VIROLOGICAL AND IMMUNOLOGICAL STUDIES ON SARS-COV-2 IN BULGARIA (2020-2022)

Iva Christova, Ivan Ivanov, Ivaylo Alexiev, Lubomira Grigorova, Anna Gancheva, Reneta Dimitrova, Ivan Stoikov, Deyan Donchev, Nely Korsun, Ivelina Trifonova, Radoslava Emilova, Iva Trifonova and Maria Nikolova

National Center of Infectious and Parasitic Diseases, Sofia

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Emerging SARS-CoV-2 led to an unprecedented pandemic related to the challenges facing national health systems, social systems and the global economy. The aim of the study was to analyze the evolving mutations and viral sublineages of SARS-CoV-2 in Bulgaria by using whole genome next generation sequencing (NGS), as well as to estimate viral load kinetics and the long-term SARS-CoV-2 specific immunity. Whole genome NGS of SARS-CoV-2 was performed on samples from randomly selected individuals by using a modified ARTIC v3 tailed amplicon method and sequenced using Illumina MiSeq. The reads were trimmed, quality filtered and full genomes were assembled in Geneious Prime 2021.1. Lineage assignment was performed with Pangolin COVID-19 lineage classification tool and the mutation pattern was analyzed by using NextClade. The phylogenetic analysis was conducted using IQ-TREE2, USHER and outbreak.info. SARS-CoV-2 viral load was measured by quantitative Realtime RT-PCR. The specific immunity was evaluated 12 months after a positive PCR test (A, n = 37) or completed immunization with a vector anti-SARS CoV-2 vaccine (B, n = 36). RBD-specific IgA and IgG (Euroimmune ELISA), and (S1+N)-specific T-lymphocytes (T-Spot Covid, Oxford Immunotech) were determined.

Epidemiological data were linked to the sequencing results to analyze and track the development of the pandemic against the background of pandemic waves, vaccination coverage and the spread of different virus variants and their sub-variants in the country. Various variants and subvariants of the emerging SARS-CoV-2 were identified, including the Wuhan variant, as well as variants of concern (VOCs), including: Alpha (B.1.1.7), Beta (B.1.351), Delta (B.1.617.2), Omicron (B.1.1.529) and a whole host of their sub-variants. Evolving mutations in the spike protein facilitating the spread of SARS-CoV-2 in the population were identified. Relation between the time from the onset of COVID-19 and the viral load was found. There was a tendency for slower reduction in viral load in more severe COVID-19 cases. RBD-specific IgA and IgG were detected in 86% and 74% of group A, (average index 5,91 and 5,45), and 92% and 100% of group B samples (7.1 and 10.6). SARS-CoV-2-specific IFNγ+T cells were present in 76 % of group A (36 SFC/well) and 64% of group B samples (14 SFC/well). The intensity of T-cell responses in both groups was significantly higher after hybrid exposure: 48 vs. 16, and 19 vs. 9 SFC/well.

SARS-CoV-2 variants with enhanced disease transmission and the ability to evade antibodies represent a threat to pandemic control and mitigation efforts. Our findings indicate the need for careful monitoring of the viral genome regions targeted by the vaccines and analysis of the postvaccination immunity. Immunity studies indicate that frequent low-dose exposures to the evolving virus boost existing natural and/or post-vaccinal immunity. The unexpectedly high levels of RBD-specific IgA 12 months after exposure correspond to a particular epidemiologic situation in Bulgaria. Hybrid exposure augments specifically the long-term T-cell response to SARS-CoV-2. These issues draw attention to further research in favor of public health for the rapid response to the COVID-19 pandemic.

Keywords: sequencing, mutations, epidemiology, immunity, viral load

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Covid3

CHALLENGES OF SARS-COV-2 DIAGNOSTIC TESTS

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The diagnosis of COVID-19 is confirmed by detecting viral RNA, antigens and/or antibodies. Nucleic acid amplification tests (NAAT) in nasopharyngeal specimens are excepted as the reference method. As a result of urgent need for a rapid and high-throughput tests, different modifications were made to the available methods. Extraction-free PCR became the preferred assay in some countries such as Turkey. Another alternative is the self-testing with antigen detecting simple and rapid assays. Antigen assays cannot replace NAAT due to low sensitivity, however their high accuracy in samples with high viral load make them a method for detecting infected patients at high risk of transmission. Serological methods detecting antibody response cannot be used for the diagnosis of active infection however they are of great importance for surveillance. Neutralizing antibody response helps to understand the natural and vaccine-induced immunity. Classic neutralization assays are labor-intensive and require biosafety level 3 conditions due to use of live virus. The development of surrogate neutralization assays has allowed vaccine performance studies to become widespread.

This talk will discuss the challenges faced in the use of diagnostic tests during the COVID-19 pandemic.

Covid4

SARS COV 2 VIRUS: RATIONALES OF INTRODUCTION OF COCKTAIL ANTIBODY

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Coronaviruses (CoV) were identified as human pathogens in the 1960s. They are enveloped positive stranded RNA viruses in the order of *Nidovirales*. With their characteristic surface, the virions have a crown-like appearance under the electron microscope, which is why the viruses are named 'corona', meaning crown. Coronaviruses have been identified in various animal hosts (e.g. bats, camels & dogs). At least seven coronavirus species are known to cause disease in humans. On 31 December 2019 it was reported the first case of Covid-19 case and on 11 March 2020 WHO declares pandemic.

Monoclonal antibodies (mAbs) have revolutionized the treatment of several human diseases, including cancer and autoimmunity and inflammatory conditions, and represent a new frontier for the treatment of infectious diseases. In the last 20 years, innovative methods have allowed the rapid isolation of mAbs from convalescent subjects, humanized mice, or libraries assembled in vitro and have proven that mAbs can be effective countermeasures against emerging pathogens.

During the past year, an unprecedentedly large number of mAbs have been developed to fight coronavirus disease 2019 (COVID-19). Lessons learned from this pandemic will pave the way for the development of more mAb-based therapeutics for other infectious diseases

CoV-2: nAb cocktail derived from a large pool of candidates: Convalescent plasma from previously infected patients & Humanised mice challenged with SARS-CoV-2 spike protein. Introductions of cocktail antibody reduce the risk of resistance to nAbs.

- Combining nAbs that target different regions of the spike protein increases the likelihood that the cocktail will retain its function even if one antibody is affected
- Antibody cocktails represent a higher resistance barrier, but are not a definitive way of preventing resistance
- Current data support the rationale of cocktail therapy as a strategy to minimise risk of resistance.

Covid5

VACCINATIONS, INFECTIONS AND REINFECTIONS: CAN WE BREAK THE CYCLE OF SARS-COV-2?

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Vaccination and non-pharmaceutical interventions (face masks, social distancing and air ventilation of indoor spaces) form the main pillars of our armamentarium against SARS-CoV-2 at present. COVID-19 mRNA vaccines that were developed in a record time of just over a year have initially shown 95% effectiveness at preventing severe illness in all age groups, independent of comorbidities. A third dose enhanced protection against severe disease and essentially completed the original 2-dose scheme, the narrow interval between doses of which (21 or 28 days, depending on the chosen vaccine) was dictated by the pandemic emergency. A fourth mRNA vaccine dose was recommended for people 50 years of age or older and special categories of patients (e.g. immunocompromised subjects) during the Omicron wave of infections that heralded a turning point in the COVID-19 pandemic. Reinfections, which should be anticipated from viruses like SARS-CoV-2 that infect mucosal surfaces without a viremic phase, have become more common with the advent of Omicron and its many subvariants. The severity of these reinfections, including the risk of hospitalization and death following reinfection compared to primary infection as well as the effectiveness of vaccination boosters to reduce reinfections is still under investigation. The enhancement of our arsenal with the newly approved oral antivirals (Paxlovid and Molnupiravir) - and those that are likely to follow – might help break the Sisyphean circle of vaccine boosters, decreasing humoral immunity, infections and reinfections, and shift the pandemic landscape to a new normalcy of coexistence with SARS-CoV-2.

Covid6

GENERATION AND CHARACTERISATION OF MOLECULAR TOOLS FOR THE STUDY OF HOST IMMUNE RESPONSES IN SARS-COV-2 INFECTION

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Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) belongs to the family of beta coronaviruses and is responsible for the ongoing COVID-19 pandemic. SARS-CoV-2 may cause asymptomatic infections, as well as mild symptoms escalating to severe lung and heart complications that can be fatal in some cases. The virus genome encodes four structural proteins; Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) that conjointly compose the viral capsid. SARS-CoV-2 enters the host cells through binding of S to angiotensin-converting enzyme 2 (ACE2) surface receptor. The present study aims to identify novel immunomodulatory factors activated upon SARS-CoV-2 entry and endocytosis using pseudotyped viruses expressing SARS-CoV-2 S protein and SARS-CoV-2 virus-like particles (VLPs). VLPs are multimeric complexes composed through spontaneous autoassembly of the four structural proteins when co-expressed intracellularly and can mimic the conformation of the natural capsid. Upon transduction with VLPs SARS-CoV-2 S enters the host cell, triggering a strong immune response followed by a cytokine storm. Vesicular Stomatitis Virus (VSV)-based pseudotyped viral stocks expressing GFP were generated and tittered, taking into advantage the unique property of the promiscuous VSV G surface glycoprotein to be exchanged for SARS-CoV-2 S protein, thereby producing replication-defective VSV-S pseudoviruses. The levels of SARS-CoV-2 S protein incorporation into the VSV particle were assessed by immunoblotting. Considering that attachment of the VSV-S pseudoviruses on the cell surface may be related to ACE2 protein expression, we screened four different cell lines (kidney embryonic HEK-293, hepatoma Huh7.5, intestinal Caco-2 and lung Calu-3 cells) for ACE2 levels by Western blot. Then, we assessed VSV-S pseudovirus infection in these cell lines by measuring S protein expression by Western blotting and GFP intensity by confocal microscopy. The matrix metalloproteinase inducer protein CD147 may also promote SARS-CoV-2 entry according to recent findings. Therefore, we have constructed and validated a stable Huh7.5 cell line overexpressing CD147, in order to investigate whether it affects VSV-S and VLP entry. Finally, we have generated VLPs by co-transfecting expression plasmids coding for the SARS-CoV-2 structural proteins in HEK-293 cells and isolating the particles on sucrose gradients. The new tools are expected to assist with the delineation of downstream SARS-CoV-2 entry and endocytosis-triggered immunological events.

Keywords: SARS-CoV-2, VLPs, entry, pseudotyped viruses, CD147.

Covid7

ACCURACY COMPARISON BETWEEN ELISA AND RAPID ANTIBODY TESTS IN THE DIAGNOSTICS OF SARS-COV-2 INFECTION

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The advent of SARS-CoV-2 imposed the necessity of adjusting already existing diagnostic methods or developing new and more accurate methods for detecting the virus. For this reason, several different types of methodologies were used. Rapid nanoparticle tests, real-time polymerase chain reaction (RT PCR), enzyme-linked immunosorbent assay (ELISA), and Loop-mediated isothermal amplification (LAMP) were mainly used to diagnose the infection, and the results of these methods were included in official data and statistics. Rapid serological tests have become widespread, especially in the first year of the pandemic. Manufacturers mainly determined the specificity and sensitivity of their products in comparison to Real-Time PCR, which is a highly specific and sensitive molecular method, considered the gold standard for diagnosing SARS-CoV-2 infection. Nonetheless, there are independent reports of lower accuracy of the rapid serological tests than the one estimated by their manufacturers, which further inclines the necessity for additional research on the matter. The aim of this study is to determine what is the actual specificity and sensitivity of rapid antibody tests in comparison to ELISA - the gold standard for serological tests, and whether they are relevant enough for the routine diagnosis of SARS-CoV-2 infection? In order to provide a definitive answer to these questions, we took into consideration the principle of action of the two types of methods and examined 369 samples with double recombinant ELISA and a rapid antibody test with gold nanoparticles. The results of our study revealed that the specificity of the rapid antibody test with gold nanoparticles is 100% and their sensitivity is 76%. To conclude ELISA has higher sensitivity than rapid antibody tests. However, due to their fast results, rapid antibody tests are a sufficient diagnostic method for SARS-CoV-2 infection.

Keywords: ELISA; rapid antibody tests; efficacy comparison, nanoparticles

General and Applied Microbiology

GAM1

PROFILING THE ABYSSO-HADAL PROKARYOTIC COMMUNITIES OF THE KURIL KAMCHATKA TRENCH BY A METABARCODING APPROACH

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The Kuril–Kamchatka Trench (KKT, maximum depth 9604 m), located in the NW Pacific Ocean, is among the top seven deepest hadal trenches. The current work was aimed at getting the first in-depth investigation of its abysso-hadal prokaryotic communities, being the microbial diversity of this deep-sea environment is practically unknown. Eighteen water samples for the metabarcoding survey (V5–V6 hypervariable regions of the 16S rDNA) were collected in various abysso-hadal areas within the KKT region between 16 August-26 September 2016 by the deep-ocean Research Vessel *Sonne* (Germany), during the German-Russian expedition KuramBio II (Kurile-Kamchatka Biodiversity Studies II). The sampling involved different sites within the trench and in the nearest zones of the adjacent abyssal plan (depth 5146-9540 m). The samples, collected by a multicorer and representative of the benthic boundary layer, were taken from the water overlaying (~30 cm) of the cores. *Proteobacteria* (56.1-74.5%), *Bacteroidetes* (6.5-19.1%), and *Actinobacteria* (0.9-16.1%) were the most represented bacterial phyla over all samples, whereas *Thaumarchaeota* (52.9-91.1%) was the most abundant archaeal phylum. At all depths, the most abundant microorganisms were chemolithotrophic archaea and heterotrophic bacteria, which did not show a distinctive zonation but were detected at all depths. In fact, the archaeal diversity was highly represented by the ammonia-oxidizing *Nitrosopumilus*, and the potential hydrocarbon-degrading bacteria *Acinetobacter*, *Zhongshania*, and *Colwellia* were the main bacterial genera. The α-diversity analysis evidenced that both prokaryotic communities were characterised by low evenness, as indicated by the high Gini index values (> 0.9). The β-diversity analysis (Redundancy Analysis) indicated that, as expected, the depth significantly affected the structure of the prokaryotic communities. The co-occurrence network revealed seven prokaryotic groups that covaried across the abysso-hadal zone of the Kuril–Kamchatka Trench. Among them, the main group included the most abundant archaeal and bacterial OTUs (*Nitrosopumilus* OTU A2 and OTU A1; *Acinetobacter* OTU B1), which were ubiquitous across the trench. The current work allowed portraying the bacterial and archaeal communities of the KKT abysso-hadal zone, which were never investigated before, evidencing that this extreme environment harboured communities that differ from those of other marine trenches.

Keywords: Kuril Kamchatka Trench, abysso-hadal prokaryotic communities, benthic boundary layer, metabarcoding, Kurambio II.

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GAM2

CAN INDUSTRIAL MICROBIOLOGY PROVIDE SUSTAINABLE ALTERNATIVES TO OUR SLAVE-LIKE DEPENDENCE ON FOSSIL RESOURCES?

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The great challenge of the 21st century is to provide sustainable solutions to mitigate climate change. This challenge could be met in part by the transition from a fossil-based economy to a renewable carbon based economy. This bio-based economy is however difficult to implement because it is based on the exploitation of biological systems used as factories for bioproduction. Unlike chemical processes, biological systems are very complex, inefficient, and difficult to manage. Therefore, a new generation of Industrial cell factories that are able to utilise all available renewable carbon sources (from CO2 to sugars) and convert them into bio-based products at optimal carbon conservation (*eg* yield) -is required. This carbon conservative –controllable- metabolic network shall be structured to provide the necessary requirements for cellular resource, while optimally feeding the bioproduction pathways to achieve maximum titer, productivity, and yield, making the whole bioprocess economically viable. This unprecedented goal will be achieved by an important rewriting of the carbon metabolic networks and by dynamic control of the bioproduction pathways following the principle of orthogonality, each of which can be achieved independently to facilitate the building of efficient cell factories combining the latest state-of-the-art technologies, including genome engineering, evolutionary engineering, and computational tools for pathways design. This high-risk but high beneficial project aims to lay the foundations for the Bio-industry of the future and shall meet society's expectations in terms of environmental performance, sustainability and employability. In my talk, I will elaborate on the two assets of industrial biotechnology, which are systems and synthetic biology, taking a specific case study in my lab to show that Industrial microbiology could bring sustainable and economically profitable solutions to overcome our fossil resource dependency.

GAM3

PHYSIOLOGICALLY BASED *IN VITRO* MODEL TO PREDICT THE PROBIOTIC POTENTIAL OF DIFFERENT LACTOBACILLUS STRAINS IN COMBINATION OF NATURAL BIOACTIVE SUBSTANCES

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The assimilation of prebiotic oligosaccharides and their metabolism by the beneficial microflora of the gastrointestinal tract is both an individual factor due to the personal nature of the human microbiome and is also influenced by specific ecological factors in which the community of individuals is found. The study of the possibilities for prevention of dysbiosis in the gastroenterology tract, as well as recovery of existing dysbiosis through the application of probiotics/prebiotics and symbiotics is a key factor in maintaining the health status of the person. Due to the limited possibilities for applying of an in vivo experiments with the participation of volunteers, various models of in situ analyzes in conditions simulating in vitro GIT systems are applied. Data from such experiments would provide to us much more realistic information than strictly laboratory experiments, about the interaction of probiotics and prebiotic oligosaccharides under conditions of GIT, including their uptake, metabolism and production of metabolites by the probiotic bacteria.

Oligosaccharides promote growth and development of lactic acid bacteria (LAB). Hypothetically, those LAB in the host GIT with their specific enzymes for utilization of prebiotic substrates and proper transport systems has potential to compete with other microorganisms from complex community from intestinal tract. The utilization of different oligosaccharides by probiotic strains from Lactic acid bacteria have activated different bacterial enzymes. For example XOS by Lactobacillus strans requires the action of three important enzymes: β-xylosidase [EC 3.2.1.37], exo-oligoxylanase [EC 3.2.1.156] and α-L-arabinofuranosidase [EC 3.2.1.55]. When cultivated on different oligosaccharides, the studied strain produced different amounts of lactic, acetic and butyric acid. Profile of organic acids and SFCA during the metabolism of different oligosaccharides was distinguished drastically compare with those of lactulose. The studies on the influence of different oligosaccharides intake on the lipids of rat liver plasma membranes showed that oligosaccharides display various beneficial effects for the host organism, which are probably specific for each used type of prebiotic. The utilization of different types of oligosaccharides may help to explain the ability of Lactobacillus strains to compete with other bacteria in the ecosystem of the human gastrointestinal tract.

Keywords: prebiotics, oligosaccharides, dysbiosis, in vitro GIT system

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GAM4

MECHANISM OF ACTION OF ANTIMICROBIAL PEPTIDES FROM THE MUCUS OF THE SNAIL *CORNU ASPERSUM*

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Natural antimicrobial peptides present new opportunities to inhibit the growth of pathogenic microorganisms and have a broad spectrum of antimicrobial action. In recent years, the antibacterial activity of the mucus of garden snail Cornu aspersum has been proven both in vitro and also in vivo in a number of clinical studies. The primary structure of newly identified antimicrobial peptides (AMPs) in the mucus on the basis of their MALDI MS/MS spectra has been established and their antimicrobial activity against Escherichia coli and Bacillus subtilis has been investigated.

Understanding of the mechanism of action AMPs on bacterial cells requires detailed knowledge of how AMPs interact with bacterial membranes. Based on large-scale simulations we hypothesize the multistage nature of the antibacterial activity of peptides and the formation of mixed peptide clusters as a transport and concentration agent to deliver the active ingredients to the target bacterial membrane. Stepping on the simulation results, the antimicrobial activity of a number of two- and three-component peptide mixtures was probed and confirmed experimentally. The antibacterial test confirmed the inhibitory effect and the synergistic effect of different combinations of peptides against E. coli 3584 and B. subtilis. Changes in bacterial structure and metabolic activity, investigated by SEM, fluorescence and digital image analysis, present strong inhibitory effects of these AMPs in superficial and deep inoculations of *E. coli*.

The clinical efficacy of these compounds has been investigated through in vivo trials in both animal models and humans. Wound studies show the presence of bacterial and fungal infections, which are a major complication of diabetic wounds and cause serious harm to patients. Traditional therapies and natural products have been used to stimulate the wound regeneration process with promising results. Antibacterial and antifungal activity of *C aspersa* snail mucus in patient wound isolates, as well as the regenerative effect, have been demonstrated.

Keywords: mucus of garden snail *Cornu aspersum*; antimicrobial peptides; wound healing

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GAM5

BIODIVERSITY OF MARINE FUNGI AND THEIR POTENTIAL IN BIOTECHNOLOGY

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Biodiversity in marine ecosystems is still largely unknown in particular with regard to microbial communities. This is partly due to the technical hurdles of collecting accurate samples, primarily in open areas. In addition, there is still relatively little microbiological research carried out in a systemic way. Marine mycology has received great attention in recent decades, mainly due to the biotechnological potential of marine microorganisms, but the diversity of marine fungi seems to be still largely unexplored. The marine environment is extremely complex showing several physio-chemical factors (salinity, low water activity, high concentrations of ions etc.) exerting a strong evolutionary pressure, often leading to the evolution of peculiar metabolisms. This is particularly clear in fungi that, in order to survive, must exercise some level of environmental control and whose metabolic and nutritional processes are largely extracellular. Several novel and unusual metabolites or enzymes that are not present in terrestrial strains, as well as a variety of genetic variations, are present in the marine strains of common terrestrial fungi. For example, *Debaryomyces hansenii* has a much higher number of genes in marine strains than in terrestrial ones. The marine strain of *Clonostachys rosea* IG119 was able to grow and produce chitinolytic enzymes under polyextremophilic conditions (pH and salinity). The new strain of *Mariannaea humicola* IG100, recently isolated from Posidonia oceanica leaves, cultivated in appropriate conditions, produced significant amounts of two bioactive terpenoids: terperstacin and the xenicane19-acetyl-4-hydroxydictyodiol. Both compounds showed a very good antifungal activity against toxinogenic/pathogenic species, which are of great significance in ecological, agronomical, economical and clinical fields. It is worth noting that the compounds, produced by the epiphytic fungus *M. humicola*, in accordance with the general behaviour reported for xenicanes, could exert for the host a specific defensive role. As far as we know, the xenicane 19-acetyl-4-hydroxydictyodiol was isolated previously only from *Dictyota plectens* and it was never detected in fungi. Recent research was conducted to examine the diversity of colturable marine epizoic fungi of *Pelagia noctiluca* and *Halocynthia papillosa* in the coastal region of Giglio Island (Tyrrhenian Sea); these studies are the first ones aimed at characterizing the epizoic communities of these marine animals. The fungal strains were identified by a polyphasic approach using morphological and molecular methods. The mycobiota detected showed a relatively high diversity, if compared to other epizoic fungal communities and strongly related to the substrate. The phylogenetic analyses suggested the presence of several new taxa. Strains were investigated for their growth preference with respect to salinity to discriminate marine-from marine-derived fungi. Also, the antimicrobial activity of the isolated fungi was investigated; more of the 68% of the strains, including the new taxa, produce bioactive compounds. The abundance of bioactive strains may be connected to a potential active involvement for epizoic fungi in host defence strategies. Very likely, most of the produced compounds could represent a potential for further biotechnological development, particularly considering the new taxa.

Keywords: marine fungi, epizoic communities, biodiversity, secondary metabolites, enzymes.

GAM6

LOCALIZATION IN RELATION TO FUNCTION OF SIALIDASES IN PROKARYOTES AND EUKARYOTES

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Sialidases (E.C. 3.2.1.18) are a large group of enzymes that catalyze the cleavage of sialic acids from carbohydrate components in the composition of complex macromolecules. These enzymes and their substrates occur in animals of the line Deuterostomata - from Echinodermata to Mammalia and in various microorganisms that are mainly commensals or pathogens for the animals. In vertebrates, sialidases are mainly localized in organs with intensive metabolism and a high content of sialic acids (brain, liver, kidneys, mammary gland, etc.). They are associated with vital biological processes, as evidenced by the existence of distinct types of sialidases located in different compartments of the eukaryotic cell. In mammals and in humans, they are four - lysosomal, cytosolic, plasma membrane, and mitochondrial/lysosomal. Sialidases are present in some viruses of the Orthomyxoviridae and Paramyxoviridae families. They are always located on the surface of the virus particle, determining its antigenic properties and virulence. Newly formed viruses are initially attached to sialic acids on the surface of the cell membrane. Viral sialidase cleaves the sialic acid molecule, freeing the virus to infect other cells. Most bacterial sialidases are secreted enzymes, but there are also ones incorporated into the cell membrane (*Haemophilus parasuis*, *Klebsiela pneumoniae*, *Pseudomonas aeruginosa*) or located in the cytosol (Clostridium perfringens, P. aeruginosa). Some bacteria produce two or more sialidases as isoenzymes with different localization in the cell. For example, *Streptococcus pneumoniae* possesses three sialidases, which are suggested to have distinct roles in the pathogenetic process. Two sialidases have been identified in *C. perfringens*. The first is located in the cytoplasm and is supposed to degrade short oligosaccharides that enter the cell for nutritional purposes. The second is secreted outside the cell and is related to virulence. In recent years, evidence has emerged for the presence of sialidases in many mold species. To date, the best studied is that of *Aspergillus fumigatus*, and it has been shown that the enzyme is mostly associated with the cell envelope (wall or membrane) and to a lesser extent is found in the cytosol. The localization of sialidases in representatives of mold fungi is currently a subject of an experimental study by our team within the framework of a project at the National Science Fund.

Keywords: sialidase, cell-associated localization, virulence

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GAM7

ANTIBACTERIAL EFFECT OF METAL NANOPARTICLES SYNTHESIZED FROM PLANT EXTRACTS

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Parts of plants are involved in the synthesis of various types of natural nanoparticles (NPs). Low cost and highly environmentally friendly plants are very useful for medical use. Although several chemical methods have been widely used to synthesize many different metallic NPs, these methods are strictly limited due to the introduction of some toxic chemicals on the surface of NPs and including time-consuming procedures. To address these disadvantages, the green synthesis methods have been developed to fabricate biocompatible and eco-friendly NPs. Recently, various biomolecules including DNA, enzyme, peptide, protein and plant extracts have been exploited in synthesis of biogenic NPs. Among these biomolecules, plant extracts have been receiving the considerable attention of researchers. They have several advantages over other biomolecules due to these potential reasons: (i) plant extracts are very cheap and provide large scale production, (ii) no need for special storage conditions, (iii) no risk of contamination and (iv) plant extracts are very stable against harsh conditions (elevated temperatures, wide range of pH and salt concentrations). In typical plant extract mediated NP synthesis, the extract simply is mixed with metal salts with a certain mass ratio and then the NP formation is carried out at various temperatures (from room temperature to 100 °C) at different periods of time (from minutes to hours) based on applied synthesis protocols. We have proven that plant extracts from *Cotinus coggygria, Chammomilla recutita, Calendula officinalis* and *Plantago major* are very rich in active substances, which in the presence of $ZnSO_4$ or $CuSO_4$ synthesize nanoparticles by various green methods. The formation of NPs has been proven by various spectroscopic methods and techniques, such as UV- and fluorescence spectroscopy, scanning electron microscopy, elemental analyses and etc. Synthesized CuONPs and ZnONPs show an increase in antibacterial activity against different strains of *Escherichia coli* and *Bacillus subtilis*.

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GAM8

CELL RESPONSE TO COLD STRESS IN ANTARCTIC FUNGUS *PENICILLIUM GRYSEOFULVUM*

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Filamentous fungi are an important part of the polar ecosystem. Cellular response against cold stress has been studied in various bacteria and plants but little is known about the adaptation of fungi to survive at temperatures close to negative. Short-term temperature downshift for 6 hours (at 6ºC and 15ºC temperature respectively) was applied to the Antarctic psychrotolerant fungus *Penicillium griseofulvum* to investigate the oxidative stress events caused by low temperature. Significant changes in growth, glucose consumption and ultrastructural morphology were observed. The cells' response to low temperature included changes in oxidative stress biomarkers such as an enhanced level of oxidatively damaged proteins, accumulation of reserve carbohydrates, and increased activity of the antioxidant enzyme defence. For the stress period at 6ºC temperature, the accumulation of the damaged proteins was about four times higher than the control. The levels of reserve carbohydrates in the cells presented by glycogen and trehalose also were increased. Two hours after the stress, the concentration was higher than the control with 72 % for the glycogen and 14% for the trehalose. Changes in the activity of the enzymes from the antioxidant defense system superoxide dismutase (SOD) and catalase (CAT) were observed. The increase in SOD activity at the 6th hour was more than three times higher than the control variant cultivated at optimum temperature. The synthesis of CAT was also stimulated during low temperature stress and the activity was two times higher than the control level. Investigated trends of the stress biomarkers at 15 ºC are similar but in a lesser degree. This study aimed to observe the survival strategy of filamentous fungi in extremely cold habitats and to acquire new knowledge about the relationship between low temperature and oxidative stress.

Keywords: Antioxidants, cold stress, Antarctic fungi, biomarkers

GAM9

ENZYME PROFILE OF LACTIC ACID BACTERIA STRAINS ISOLATED FROM BREAST MILK

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Mother's breast milk is considered the optimal feeding option for newborns, and the World Health Organization WHO recommends that breastfeeding continues until at least 6 months of age. The bioactive substances contained in breast milk lead to improved health and immune development of babies, with fewer cases of gastrointestinal diseases and lower infant mortality rates compared to formula-fed newborns. In addition to providing essential nutrients to the growing baby, breast milk is a source of commensal bacteria that further improve the baby's health by preventing the adhesion of pathogens and promoting the colonization of beneficial microbes in the gut. While breast milk was originally thought to be a sterile fluid and isolated microbes were thought to be pathogens, it is now widely accepted that breast milk possesses its own unique microbiome. The origin of bacteria in breast milk is the subject of much debate, but the possibility of an enteromammary pathway allowing the transfer of microbes from the maternal gut to the mammary gland is one potential pathway. The microbiome of the mother's breast milk is essential for the formation of the microbiome in the intestinal tract of the newborn. Our study aims to determine the specificity of the microbial composition of human milk and the percentage of beneficial microflora depending on the nutritional habits of mothers. The emphasis of the research is the characterization of basic enzyme activities of isolated lactic acid bacteria as potential biomarkers for absorption of oligosaccharides from a mother's breast milk. Predominant presence of heterofermentative lactobacilli found in breast milk. The activity of β-galactosidase, α-galactosidase, and fucosidase is on the one hand strain-specific and on the other hand, depends on the environmental environment and the mother's diet. The studied enzymes are of key importance both for the rapid absorption of lactose and the metabolization of the oligosaccharides contained in breast milk.

GAM10

NEW BETA-GALACTOSIDASES FROM LACTIC ACID BACTERIA

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Beta-galactosidase (EC3.2.1.23) is a glycoside hydrolase enzyme, which cleaves the O-glycosyl bond of lactose, degrading it to glucose and galactose. Beta-galactosidase also transfers a galactosyl moiety onto another molecule galactose or lactose and thus produces galacto-oligosaccharides (GOS) with prebiotic properties. We have studied a beta-galactosidase from Lactobacillus delbrueckii subsp. bulgaricus strain 43. The gene sequence consists of 3,025 of bp. We amplified the gene from total DNA via PCR, with primers specifically designed to contain restriction sites for XhoI and NdeI. These restriction enzymes were used for cloning the beta-galactosidase gene into pET-41b(+) vector with His tag. The recombinant construct was amplified into StellarTM competent cells (TakaraTM). Successful clones were confirmed by both restriction analysis and sequencing/blast analysis in the NCBI database. These clones were used for the transformation of E. coli BL21 (DE3) via electroporation with 1.8 kV for 5.7 ms using BioRadTM MicroPulser. Competent BL21 cells were produced after washing with ice-cold 10% glycerol according to the instructions of BioRadTM. Transformed cells were isolated on LB agar with Kanamycin. They were cultivated in a liquid medium until OD(600 nm) reached 0.9 and then induced with 1 mM IPTG for 20h. Cultivated cells were harvested by centrifugation and sonicated at 20 kHz with 35W. The recombinant protein was isolated, visualized on SDS-PAGE as a band of approximately 120 kDa, and purified, using His GraviTrapTM columns with Ni Sepharose (GE HealthcareTM). Equilibration buffer and the sample contained 20 mM imidazole. So we obtained partly purified beta-galactosidase. The most successful elution was achieved with 150 mM imidazole.

Keywords: beta-galactosidase, *Lactobacillus delbrueckii subsp. bulgaricus*, *Escherichia coli* BL21, DNA cloning

GAM11

CLONING AND EXPRESSION OF INULINASE GENE IN 2,3-BUTANEDIOL PRODUCING *BACILLUS LICHENIFORMIS* 24

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Bacillus licheniformis 24 is known as a useful strain for bioconversion of fructose-containing feedstock for economical 2,3-butanediol (2,3-BD) production. In our previous work, in fed-batch fermentation from a total of 370 g/L fructose, *B. licheniformis* 24 produced 156.1 g/L 2,3-BD in 72 hours with a yield of 0.42 g/g and productivity of 2.17 g/L/h, which was one of the best results for 2,3-BD production from any substrate obtained to date. Inulin is a renewable and relatively cheap carbon source applied in microbial fermentations. It is a poly-fructan composed of fructose units linked via β (2→1) bonds and a single glucose moiety at the reducing end. The cloning and expression of heterologous inulinase in *B. licheniformis* 24 would enhance its native glycoside-hydrolase activity and would intensify the microbial process for 2,3-BD production from inulin. For this purpose, the gene inu of L. paracasei B41 (3654 bp in length and encoding a protein of 1214 amino acids) was chosen. The DNA fragment containing the inu gene was amplified using total genomic DNA of *L. paracasei* B41 and primer pair INF (5'atggatgaaaagaaacattacaagatg3') and INR (5'ttagactcgcttcacccgcctc3'). Then, the gene was cloned in pJET1.2/blunt vector and extracted after restriction with XbaI and XhoI. A truncated version of the gene (2.2 kb) lacking the four Big3 domains responsible for cell wall anchoring, was amplified via PCR with a different pair of primers with introduced restriction sites InuF_Xho and InuR_Xba. Both genes were cloned into a pBE-S shuttle vector (5938 bp) for *E. coli* and *Bacillus*. The recombinant constructs were transformed into E. coli StellarTM competent cells (Takara), purified in sufficient amounts, confirmed by restriction analysis, PCR, and sequencing, and introduced into Bacillus licheniformis 24 via electroporation. The electro-competent bacilli were produced after repeated washing in electroporation buffer (0.5 M sorbitol, 0.5 M mannitol, and 10% glycerol). LB medium with 0.5 M sorbitol and 0.38 M mannitol was used as a recovery medium. The inulinase activity of selected clones was tested on an agar medium supplemented with 1% HD inulin. Iodine staining was used to determine the hydrolysis zones. B. licheniformis clones containing a 3.6 kb gene possessed much greater activity, with a diameter of 2.5 cm hydrolysis zones. In contrast, the truncated variant (2.2 kb gene) showed reduced activity, thus revealing that Big3 domains are also important for enzyme function. Further research will combine the recombinants' enhanced inulinase activity with the strain's producing abilities to obtain a microbial cell factory for simultaneous inulin hydrolysis and fermentation.

Keywords: 2,3-Butanediol, inulinase, *Bacillus licheniformis*.

GAM12

OPTIMIZATION OF THE ENZYME PRODUCTION OF MUTANT GLUCOSYLTRANSFERASE URE 13-300 IN RECOMBINANT STRAIN *ESCHERICHIA COLI* BL21

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Leuconostoc mesenteroides URE 13 strain produces glucosyltransferase enzyme with a high molecular mass of about 300 kDa. The gene encoding the enzyme glucosyltransferase URE 13-300 was cloned and expressed in *Escherichia coli* BL21. The enzyme is an α-transglucosylase, part of the GH70 family, synthesizing insoluble glucan with α-1,6 and α-1,3 linkages. Site-directed mutagenesis with substitution of one amino acid was performed in order to study the changes in the synthesized products. Mutant enzyme production by recombinant strain *E. coli* BL21 in shake flasks was optimized by adjusting the fermentation conditions regarding several factors: the temperature for cultivation, biomass accumulation time, and amount of added inductor isopropyl-β-D-thiogalactopyranoside (IPTG). While investigating the correlation between accumulated biomass and enzyme production, we noted that higher enzyme activities were related to higher optical density (OD) detection prior to the addition of IPTG. The induction was set to start at an OD value between 1.6 and 1.8. The induction temperatures ranging from 14°C to 25°C were tested. The optimum appeared to be 16°C which is the same as with the initial recombinant strain. Further accumulation of biomass was much slower after induction and there was a notable decrease in enzyme activity, therefore the duration of fermentation was fixed at 16 hours. In respect of the amount of inductor added, the final concentration of 0.7 mM and 1.0 mM had almost the same effect. The highest enzyme activity of 1.3 U.mg-1 was measured. Optimization of the mutant glucosyltransferase URE 13-300 enzyme production is essential for further examination of its functional properties. The obtained crude enzyme extracts will be used for the synthesis of oligosaccharides and glucan for investigation of their structural and functional properties.

Keywords: glucosyltransferase, mutation, oligosaccharides, glucan

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GAM13

CHARACTERISATION OF GLUCANSUCRASES PRODUCING *LEUCONOSTOC MESENTEROIDES* STRAINS, ISOLATED FROM KEFIR

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In the current work, we report the isolation of 40 isolates of lactic acid bacteria from 5 different samples of the traditionally fermented milk drink kefir. The obtained isolates were screened on Mayeux, Sandine, and Elliker (MSE) agar medium supplemented with 10% sucrose at 23 °C for their ability to produce exopolysaccharides. The most significant mucous growth was observed in 5 strains, namely D5-1, D6-1, D7-1, D8-1, and D9-1. Furthermore, the ability of these strains to grow and produce exopolysaccharides was tested on MSE agar supplemented with 5% glucose, fructose, or lactose. The isolates grow well on these carbohydrate sources but differ significantly in the formation of exopolysaccharides and their texture. All five strains are Gram-positive and catalase-negative, and their cells have slightly elongated ovoid shapes and are organized in pairs and short chains. The performed 16S rDNA sequencing identified them as *Leuconostoc mesenteroides* with >95% of identity. Leuconstoc spp. is a known producer of glucan polysaccharides from sucrose by the action of glucansucrase enzymes [1]. We detected the production of extracellular and cell-associated glucansucrases after cultivation of the isolates in an MSE medium supplemented with sucrose and in situ electrophoretic assay of the supernatant and cell fractions. In the samples of all five isolates were detected bands of activity corresponding to 180 kDa glucansucrase which by size corresponds to dextransucrase [1]. One of the studied strains produces a second glucansucrase with Mw 220 kDa. The production of extracellular glucansucrases by the isolates was optimized by their cultivation at a concentration of sucrose ranging from 2% to 10%. The highest extracellular glucansucrase activity was measured for strain L. mesenteroides D6-1 at the 6th hour of the cultivation and 8% initial sucrose concentration – 2,28 U/ml. Extracellular glucansucrase preparations from the five strains were used for the synthesis of glucan polysaccharides from sucrose. Then, the synthesized glucans were subjected to enzymatic digestion with endo-dextranase from Penicillium spp. in order to be calculated the percentage of α-1,6-linkages in their structures. Of interest are the glucans synthesized by glucansucrases from strains L. mesenteroides D6-1 and D8-1 which appear to contain only 40,81% and 32,43% α-1,6-linkages that suggests the presence of other specific glycoside linkages and branched structures. This is a prerequisite for specific properties of these glucans which are yet to be assessed on a practical level.

Keywords: glucan exopolysaccharides, glucansucrases, kefir growth" 2014–2020, grant number BG05M2OP001-1.002-0005-C01, Personalized Innovative Medicine Compet

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GAM14

BACTERIOPHAGE SEED COATING AS PREVENTION APPROACH IN COMBATING *XANTHOMONAS EUVESICATORIA*-ASSOCIATED PLANT DISEASES

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Seeds are considered as the main reservoir for the distribution of phytopathogenic bacteria. In this stage of the bacterial life cycle, the cells only persist on or in plant seeds. When the environmental conditions become favorable for seed germination the bacterial cells start growing and in the cases of phytopathogenic bacteria – to switch to pathogenic behavior. Therefore, if phytopathogenic bacteria are destroyed while they are in their survival stage (seeds) the probability of developing a severe plant disease will be decreased. In this regard, seeds could be considered as suitable for examinations of different antibacterial preparations in order for future severe bacterial diseases to be avoided. The main goal of our study was to determine the ability of newly isolated bacteriophage (BsXeu269p/3) to destroy the phytopathogenic bacteria *Xanthomonas euvesicatoria* in contaminated tomato seeds. The objects of the analysis are seeds of three sorts of the plant *Solanum lycopersicum* (tomato) - "Pink Magic", "Ace" and "Ideal". The seeds were treated with phage isolate via 3 different variations. In the first, 50 seeds of each sort were soaked in phage suspension and subjected to vacuum infiltration for 50 min at 1atm. In the second variant, seeds were only soaked in phage suspension for 1h. In the third variant, the seeds were soaked in phage suspensions with added sucrose to a final concentration of 1%. The role of host bacteria in phage persistence in/on seeds after treatments was established as tomato seeds were vacuum infiltrated firstly with a saline suspension of *X. euvesicatoria* (108 CFU/mL) and subsequently vacuum infiltrated with BsXeu269p/3 suspension (108 pfu/mL). Phage-free control was included in the experiment (seeds treated only with bacterial suspension). The results showed that after the treatment of the seeds by all three variations, bacteriophages were successfully reisolated from the seeds, with the highest number of viable phage particles (104 pfu/mL) being isolated from the soaked seed (Sort Ideal) in phage suspension +1% sucrose. Comparing the obtained results for phage viability in treated seeds in the presence and absence of its specific host bacteria showed that a greater number of viable phage particles were reisolated from seeds previously treated with *X. euvesicatoria*. Each treated seed was individually tested for phage (BsXeu269p/3) persistence on its surface and the best results were observed in the treatment variants: 1/ soaking in phage suspension + 1% sucrose and 2/ vacuum infiltration with bacteriophage + its host. The percent viable phage particles isolated from those seeds was up to 90 and up to 100, respectively. The obtained results indicated that the most effective approach for seeds treatment is with phage suspension + 1% sucrose. Seed treatment with bacteriophages could be considered as promising preventative approach in combating *X. euvesicatoria* associated bacterial diseases in tomato plants.

Keywords: seed antibacterial treatment, bacterial spot disease, phage biopesticide, *Xanthomonas euvesicatoria*

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GAM15

SESQUITERPENE LACTONES WITH PROMISING INHIBITORY EFFECTS ON BACTERIAL QUORUM SENSING AND BIOFILM FORMATION IN GRAM NEGATIVE AND GRAM-POSITIVE BACTERIA

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In the new antibiotic era, the exponentially increasing number of multiresistant bacterial strains becomes the main global health problem. Together with this, there is scientific interest in novel alternative ways to inhibit bacterial virulence. One particularly attractive and promising way is to interrupt the main regulatory mechanisms of bacterial virulence determinants known as Quorum sensing (QS). The inhibition of QS signals will be a novel way for screening of more effective metabolites of different medicinal plants put a key role in next-generation antimicrobials in the resistance battle. The aim of the present study is to check the possibilities of purified sesquiterpene lactones (Asteraceae family) to inhibit the mechanisms of bacterial quorum sensing and biofilm formation. The model bacterial strains used in the study were *E. coli* 25922, *S. aureus* 29213, *C. violaceum* 30191, *P. aeruginosa* PAO1. Biofilm formation from Gram negative and Gram positive strains was evaluated by the Crystal violet assay. The anti-quorum sensing activity was determined by assessing the violacein production of *C. violaceum* in the presence of lactones. The results from anti-biofilm activity showed that all sesquiterpene lactones have strong anti-biofilm activity. After the extraction of the pigment violacein, it was found that five of them inhibit pigment production, namely 1β-hydroxyarbusculin, parthenolide, tatridin B, xerantholide, and arnifolin. In order to shed light on the intimate anti-QS activity of the studied lactones, we performed an „Induced Fit" docking study. In this, we test the ability of studied lactones to form complexes with the active site of the ligand-binding domain of CviR - a cytoplasmic DNA binding transcription factor of *Chromobacterium violaceum* 30191. The obtained results show the potential of such lactones in the inhibition of QS signals.

Keywords: Quorum sensing, sesquiterpene lactones, Astereaceae, biofilm, docking

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GAM16

INULA BRITANNICA L. EXTRACTS AS NATURAL INHIBITORS FOR CONTROL OF BACTERIAL VIRULENCE FACTORS

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Nowadays, it is necessary the discovery of new targets for the inhibition of microbial pathogenicity, without stimulating microbial resistance. One of the most novel anti-virulence strategies is to interrupt the cascade of the Quorum sensing (QS) system. Briefly, QS changes gene expression and thus controls virulence, pathogenicity, antibiotic resistance, and biofilm formation. This high level of organization helps the microbial population recognize and respond to different environments.

Each step in the QS mechanism could be a good target and result in the inhibition of pathogenicity. One of the most attractive biomolecules, which could be used for this purpose, is natural plant metabolites. In this work, different extracts from *Inula britannica* L., the well-known medicinal plant, were investigated as natural virulence inhibitors through different methods such as cristal violet assay, quantifying pigment production, swimming, swarming, and twitching motility. Biofilm reduction was estimated by a crystal violet assay in four pathogenic bacterial strains - *E. coli* 25922, *S. aureus* 29213, *C. violaceum* 30191, *P. aeruginosa* PAO1. Different methods were used to study the effects of extracts on QS-controlled virulence factors such as pigment production, swimming, swarming, and twitching motility. The biofilm formation ability was stronger by IBr1 and IBr1-SL in all of the tested strains. This correlation was confirmed by fluorescence staining analysis, including reduction of viability and probably loosening of biofilm matrix in the treated samples. Scanning electron microscopy investigations in treated bacteria imply elongation in cell size with filamentous forms and disorder in cell division with an inability to form bacterial septa. In addition, their anti-virulence potential was monitored by inhibition of purple pigment production in *C. violaceum*.

Keywords: Quorum sensing, virulence factors, plant extracts, *Inula Britannica* L.

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GAM17

INHIBITORY CAPACITY OF LACTOBACILLUS POSTBIOTICS ON BIOFILM FORMATION AND QUORUM SENSING ACTIVITY

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The Quorum sensing (QS) system regulates the expression of different virulence factors in pathogenic bacteria. Its inhibition by newly characterised bioactive molecules should be one of the novel strategies for combating pathogens. In the present work, we assessed for QS inhibitory activity produced postbiotics by candidate-probiotic lactobacilli. *Chromobacterium violaceum* is a Gram-negative, facultative, anaerobic beta-proteobacterium, forms violet colonies. The color is due to the release of the water-insoluble pigment violacein, encoded by the vio operon, whose expression is controlled by quorum-sensing signals. The QS induces virulence factors and modulates bacterial behavior, including development and biofilm formation. The aim of the study was to test the capacity of lactobacilli to inhibit major virulence factors - biofilm formation and pigment production with *C. violaceum* pathogenicity. The results showed that biofilm capacity was significantly reduced by filtered non neutralized cell-free supernatants and cell-derived fragments (lysates) from lactic acid bacteria (LAB) isolated from different habitats. Their anti-virulence and QS-inhibition efficacy were evaluated by quantification of the synthesized pigment violacein and Agar diffusion assays. The LAB samples showed inhibitory activity of violacein production in contrast with the control probe. Further validation of purified active postmetabolites has to be done and it is still in progress.

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GAM18

MIXED POLYMERIC MICELLES LOADED WITH CIPROFLOXACIN FOR THE TREATMENT OF *S. AUREUS* BIOFILM INFECTIONS

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The evidence for the role of biofilms in chronic, persistent, and recurrent infections necessitates the development of novel approaches in the fight against them. Biofilms are consortia of microorganisms adherent to various surfaces including host tissues. Biofilm bacteria are embedded in extracellular polymeric substances (EPS). EPS protects the microorganisms from environmental hazards including access of antibacterial substances. The structural and functional characteristics of biofilms determine the high drug tolerance of biofilm infections. This requires the development of novel drug-delivery systems capable to overcome the barrier created by the EPS and release the drug they carry directly to the biofilm bacteria. The aim of the study was to develop and test novel polymeric carriers designed for the delivery of ciprofloxacin. Mixed polymeric micelles of different composition based on poly(2-(dimethylamino)ethyl methacrylate)-b-poly(ε-caprolactone)-b-poly(2-(dimethylamino)ethyl methacrylate) and poly(ethylene oxide)–b-poly(propylene oxide)–b-poly(ethylene oxide) triblock copolymers were investigated. Their reduction effects on the biomass (CV assay) of mature biofilms of *S. aureus* 29213 confirmed the antibiofilm efficacy of the novel systems. Together with this, the significant suppression of the biofilm metabolic activity (Alamar blue reduction assay) by the CF-loaded micelles supported the delivery of the drug to the biofilm bacteria. Murine skin explants were pre-treated with the micelles (pure or loaded with CF) and then infected with S. aureus 29213. This was followed by tests for the evaluation of NO2 and TNF-α production. Significant differences were registered between the data obtained for the skin explants pre-treated with the unloaded and the CF-loaded micelles. This again is in support of the successful drug delivery by the novel polymeric carriers.

Keywords: *S. aureus* biofilm, Polymeric micelles, Drug delivery

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GAM19

CELLULAR EFFECTS ON *S. CEREVISIAE* OF CHITOSAN-FERRIC OXIDE NANOCOMPOSITES DOPED WITH Cu^{2+}

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Together with the importance of *S. cerevisiae* in food industries, it is as well used as a model microorganism for testing the antifungal potency of natural and synthetic substances including nanocomposites. An emerging direction in the design of novel nanostructured materials is, dependent on the aimed application of the material, to modulate the bio-active characteristics by the inclusion of additional ions to metal nanoparticles. The aim of the present study is to check the antifungal potential of hybrid chitosan-ferric oxide CS/Fe2O3 nanocomposites doped with different amounts of Cu2+ ions, (0, 0.1 M, 0.2 M, and 0.3M) using *S. cerevisiae* NBIMCC 3017 as a model fungal strain, and to examine the effects of the substances on the cellular level. MICs of the nanocomposites were determined by microdilution in YP broth in the presence of Alamar blue as a viability marker. The minimal fungicidal concentration was established by agar plating. To examine the mechanisms of action of the nanocomposites on the yeast cells, we used a treatment milder than directly killing them, and the yeasts were incubated in the presence of 1/2 MIC for various time intervals. Under these conditions, the data from several applied methodologies for microscopic staining indicated that while the yeasts did not stop reproducing, a variety of cellular pathologies accumulated in the population. To check viability, methylene blue staining was applied. Following the effects with time, the early stress visualized by the increased number of colored yeast cells increased from hour 1 to hour 3. This damage appeared to be stronger in the Cu2+-containing nanocomposites. On hour 24, the per-cent of colored cells was significantly reduced and the differences between the four samples were reduced. This was probably due to the reproduction of comparatively unaltered cells. We further examined in more detail this apparent adaptation of the yeast population to the toxic action of the nanocomposites. Light and scanning electron microscopy revealed the occurrence on hour 24 of cell chains and giant cells. Live/Dead staining by the combination of two DNA markers indicated increased cell envelope permeability of the giant cells. Phalloidin-TRITC staining showed a dramatic increase in F-actin accumulation in these large cells. Calcofluor white staining indicated irregular cell division as well as an incomplete division of the yeasts organized in cell chains. The significant presence of various cell alterations in the yeast populations treated with the sub-MIC amounts illustrated the cellular effects of the tested nanocomposites and confirmed their antifungal potency.

Keywords: Antifungal, chitosan-ferric oxide CS/Fe2O3 nanocomposites doped with Cu2+, *S. cerevisiae*

GAM20

CANDIDATE- PROBIOTIC STRAINS FROM TRADITIONAL BULGARIAN PRODUCT "KATAK"

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"Katak" is a traditional fermented milk product with a specific taste. The autochthonous microbiota of katak is responsible for its unique sensory properties and long shelf life. However, little is known about its beneficial properties. With this aim, 14 newly isolated lactobacilli from a home-made sample of katak were characterized for functionality and safety. A broad spectrum of activity, including activity against food-associated pathogens and toxigenic fungi, was revealed. The inhibitory activity was confirmed in a model system, simulating in vitro gut conditions. The strains identified as *Lactoplantibacillus plantarum* showed high capability for prebiotics utilization. A candidate-probiotic strain was selected, which combines functional and technological characteristics. The *L. plantarum* isolate completed EFSA's requirements for safety, based on a spectrum of antibiotic susceptibility. The combination with technologically relevant caseinolytic activity and good growth in dairy and non-dairy matrics makes strains promising for the development of new functional foods.

Keywords: katak, probiotics, antibacterial, antifungal, functional food

Acknowledgment: This work was supported by the National Science Fund at the Ministry of Education and Science, Bulgaria approved by Research Grant № KP-06-OPR 03/16 "Probiotics and health – mode of beneficial action of selected Bulgarian strains of lactic acid bacteria".

GAM21

PLANT-ASSOCIATED LACTIC ACID BACTERIA – POTENTIAL FOR APPLICATION

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The application of lactic acid bacteria, isolated from alternative non-dairy sources as carriers of various metabolic activities, has been of great interest in recent years. The aim of the present study was to prove the potential for the application of plant-associated lactic acid bacteria (with enhanced physiological and biochemical characteristics and proven resistance to plant extracts) used for the preparation of new fermented milk products. The growth and fermentation characteristics of strains *Lactobacillus rhamnosus*, *Streptococcus thermophilus*, *Lactococcus lactis* and *Enterococcus faecium*, isolated from the medicinal plants *Panax ginseng* C. A. Meyer, *Salvia ringens* Sibth. & Sm., *Salvia blepharophylla* Brandegee ex Epling and *Salvia scabiosifolia* Lam. were studied – lactose utilization, synthesis of lactic acid, changes in pH and viability (number of the viable cells) during the fermentation process, as well as establishing the coagulation time of milk. It was also made a comparison with strains of the same lactic acid bacteria species, which were isolated from dairy products. Plant-associated lactic acid bacteria strains were proven to have better or similar fermentation and growth characteristics, in comparison to the strains of dairy origin, during cultivation in milk, which proves their potential to be included as components in starter communities, in order to obtain new fermented milk products, as well as foods with an increased nutritional and health status.

Keywords: lactic acid bacteria, fermentation characteristics, growth characteristics, application

GAM22

EFFECT OF LYSATES FROM PLANT-ASSOCIATED BACTERIA ON SECONDARY METABOLITE PROFILES OF *SALVIA RINGENS* ADVENTITIOUS ROOTS CULTURE

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Two bacteria strains have been isolated from the leaves of the fig plant (*Ficus carica* L.) and grape (*Vitis vinifera* L.). The strains were identified (16S rRNA sequencing) as *Kocuria rhizophila* (KR) and *Microbacterium paraoxydans* (MP), respectively. The bacteria have been cultivated on a nutrient broth medium and cell lysates have been prepared by using a combination of ultrasound and microwave for cell disruption, followed by autoclaving. The lysates were investigated for their effects on growth and the ability to elicit secondary metabolites production by adventitious root culture of *Salvia ringens*, cultivated in liquid MS medium. The results showed that the treatment with KR lysate (250 mg/L) significantly stimulates ($P<0.05$) the Salvia roots growth and the accumulated dry biomass reached ADB=0.37±0.02 g/L (28% increase, when compared to untreated control). Opposite, the treatment with MP lysate has no statistically significant effect on roots growth. The highest yield of rosmarinic acid (3.43±0.05 mg/L) was achieved after elicitation with KR lysate as well (26% increase, when compared to untreated control). This study demonstrates the potential application of *K. rhizophila* as an elicitor of economically important secondary metabolites, produced by the plant in vitro systems.

Keywords: elicitation, phenolic acids, rosmarinic acid, plant in vitro systems

GAM23

ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM INFANT FAECES

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Generally, lactic acid bacteria (LAB) are related to infant gut microbiota. This microbiota plays a crucial role in preventing colonization from potential pathogenic microorganisms and development the immune system. It is well known that breast milk provides infants not only with nutrients necessary for growth and development, but is the major driver for the colonization with beneficial bacteria in the gut of newborns. The main objective of this work was to perform a screening for the presence of lactic acid bacteria strains in healthy infants' faeces. LAB were isolated from faecal samples of exclusively breastfeeding infants living in Bulgaria. The samples were collected from five newborns between one to six months old. Each faecal sample, was incubated in MRS broth medium supplemented with 0.05 % (w/v) cysteine. A total of 29 colonies were selected based on different morphologies on MRS agar medium modified with vancomycin (10 mg/l). Isolated bacteria were phenotypically characterized by conventional methods including Gram stain and catalase production. For further analyses, only Gram-positive, catalase-negative and rod-shaped strains were selected. The identities of the isolates were confirmed by genus-specific PCR and MALDY-TOF-MS analyses. The isolated strains identified were Lacticaseibacillus rhamnosus (ex-Lactobacillus rhamnosus) and Limosilactobacillus reuteri (ex-Lactobacillus reuteri). The most prevalent LAB strains found in this work were L. rhamnosus - 63% of all phenotypically characterized ones. Our experiments confirmed the species L. reuteri in only one sample (10% of all investigated isolates) while L. rhamnosus was isolated from three out of the five examined samples. From all initially isolated strains, 14 lactic acid bacteria were identified to species level. Our studies on the infant feces microbiota suggested that Lactobacillus rhamnosus is dominant in terms of the genus Lactobacillus. The investigation showed the presence of LAB in 80 % of all tested infant faeces.

Keywords: LAB, infant faeces, microbiota, identification

GAM24

DIVERSITY OF THE CLOACAL AEROBIC BACTERIAL FLORA OF FREE-LIVING LIZARDS FROM IHTIMANSKA SREDNA GORA

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Current knowledge of the microbiome of vertebrates other than humans, especially non-mammals, is extremely limited. Reptiles represent 17% of all vertebrate species and lizards include about 60% of reptiles, but their accompanying microbiota has not yet been sufficiently investigated. Most studies are concentrated on a small subgroup of bacteria known as zoonotic (especially Salmonella sp.) or were done on reptiles in captivity, subject to commercial interest as pets. There is still a lack of clarity regarding microbial diversity in free-living reptile hosts. Research and comparative study on the microbiota of reptiles in their natural habitat have not been conducted to date in Bulgaria. Our research interest is focused on the isolation, identification, and characterization of cloacal aerobic bacterial flora of wild dwelling lizards. For this purpose, a place, located along the valley of the Dalbochitsa River, Ihtimanska Sredna Gora was selected. In the particular area, five syntopic species from Lacertidae (3 species), Scincidae (1 species), and Anguidae (1 species) are presented as follows: the European green lizard (Lacerta viridis Laurenti, 1768), the Common wall lizard (Podarcis muralis Laurenti, 1768), the Meadow lizard (Darevskia praticola sl Eversmann, 1834), the European snake-eyed skink (Ablepharus kitaibelii Bibron & Bory de Saint-Vincent, 1833) and the European slow worm (Anguis fragilis Linnaeus, 1758). They demonstrate differences in their microhabitat choice, dietary preferences, and width of trophic niches. Due to similar ecological requirements, the sympatric occurrence of several species of lizards is very rare. This provides an opportunity to compare their microbiota. A total of 85 specimens were captured between May and June 2022 and sampled. More than 40 isolates have been isolated to date. Classical microbiological and biochemical methods were applied for their identification. This study contributes to enriching the database of still understudied wild animals-associated microbial communities.

Keywords: bacterial diversity, cloacal microflora, reptile hosts, lizards

Acknowledgements: This work is supported by the National Science Fund of the Ministry of Education and Science, Bulgaria (Project КП –06-М51/9)

GAM25

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM BULGARIAN *EPILOBIUM PARVIFLORUM* SCHREB

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The present work analyzed the chemical composition and antimicrobial activity of the essential oil of *Epilobium parviflorum* Schreb. from two natural populations in Bulgaria. The essential oil from the aerial parts of the plant was extracted by hydrodistillation method and analyzed by GC/MS. Nineteen main compounds have been identified in the essential oil. The most abundant component is phenylacetaldehyde, making up 25.6 of 28.4% of the oil. In addition, the antimicrobial activity of the oil against Gram-positive bacterium (*Staphylococcus aureus*), and fungus (*Candida albicans*) was studied. The tested EOs demonstrated moderate activity against selected pathogens. In conclusion, E. *parviflorum* can be considered as an herbal antimicrobial agent.

Keywords: *Epilobium parviflorum*, chemical composition, antimicrobial activity.

Medical Microbiology

MM1

EPIDEMIC AND ENDEMIC *MYCOBACTERIUM TUBERCULOSIS* CLUSTERS IN RUSSIA: PATHOBIOLOGY AND PHYLOGEOGRAPHY

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Mycobacterium tuberculosis is important human pathogen. Its population structure (and consequently, epidemic situation with tuberculosis) has been shaped by multiple factors. Some of them are related to the pathogen itself (virulence and drug resistance), other depend on the human host (immune response, human migration) and the environment in the wide sense (TB control programs, social factors, climate, nutrition). *M. tuberculosis* includes several large phylogenetic lineages while Lineage 2 (East Asian) and Lineage 4 (Euro-American) are the most globally spread. In Russia, Beijing genotype (Lineage 2) and Latin-American Mediterranean (LAM) genotype (Lineage 4) are the most widespread and medically relevant. Their Russia-specific clones are of likely local origin and are marked with genetic homogeneity due to mass and partly forced population migration during the 20th century. Beijing genotype is spread across all country, but more prevalent in Siberia. LAM is relatively more prevalent in European Russia than in Siberia/Far East. A limited number of the epidemic clones of the Beijing genotype circulate in Russia that mostly belong to its modern sublineage. In my lecture, I will review our recent genomic and virulence studies of the medically significant genetic clusters of the Beijing and LAM genotypes in Russia and their possible impact on the neighboring countries and globally, due to human migration. I will present results of studies of pathogenetics, phylogenomics and phylogeography of (i) Russian MDR clusters of modern and ancient Beijing sublineages and (ii) Russian emerging subtypes of LAM_RUS sublineage.

The virulence and lethality of the strains were studied in C57BL/6 mouse model. The whole-genome sequencing (WGS) data were obtained using HiSeq Illumina platform or retrieved from GenBank, and submitted to bioinformatics and phylogenetic analysis. SIFT tool was used to predict whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids.

The epidemic cluster Beijing B0/W148 is strongly associated with drug resistance and spread across all country. I call it “Russian successful clone” and its spread presents the major obstacle to the national TB control program. In addition, we have recently discovered two MDR clusters within ancient Beijing sublineage, in the Asian part of Russia. Low virulent, low lethal (but MDR) Beijing 1071-32-cluster is widespread across FSU but at low prevalence with max 7% in Omsk, Western Siberia. This follows a common view that multiple resistance mutations reduce fitness and transmission. In contrast, MDR, highly lethal and hypervirulent Beijing 14717-15-cluster is predominant in Buryatia, Far East (16%), very rare outside it, not forming clusters of transmission. I define this the most lethal Russian Beijing cluster conditionally transmissible. The reasons may lie in the interplay of the human immune system and the genetic background of this strain. Its cluster-specific SNPs are in genes related to immune evasion and mycobacterial adaptation.

Our virulence and genomics results on the LAM genotypes in Russia demonstrate an inverse correlation of resistance and virulence and transmission. These features are also reflected in their geographic spread. The least virulent strain SIT254 was MDR (but only 3 drugs, 4 mutations) and the most widespread. The virulent but low lethal strain SIT266 was pre-XDR with 10 resistance mutations and was dominant only in one country (Belarus). The most virulent, more lethal strain SIT264 was drug susceptible; it was rarely spread in different places but slightly increased its circulation in the last decade. I conclude that different lineages and sublineages, and smaller more homogeneous clusters of human *M. tuberculosis* have developed their own and different ways of adaptation with humans due to different interplay of

bacterial virulence, resistance, that has also been modulated by coevolution with human immune system. Large-scale phylogenetic *M. tuberculosis* lineages are heterogeneous and medically relevant research should focus on genetically compact epidemic clusters. Personalized TB treatment should attempt considering not only drug resistance but also different virulence properties of the infecting strains.

Acknowledgements: Russian Science Foundation (grant 19-14-00013).

Keywords: *Mycobacterium tuberculosis*; whole-genome sequencing; phylogeography; mouse model; virulence; drug resistance

MM2

MULTIPLEX PCR FOR MODERN SYNDROMIC MICROBIOLOGICAL DIAGNOSIS OF SEVERE INFECTIONS

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In recent years, classical microbiological methods have gradually given way to modern, rapid approaches for syndromic testing, which mark new perspectives in the microbiological diagnosis of invasive infections. The potential for simultaneous detection of pathogens responsible for infections of the bloodstream, the central nervous system, the respiratory, and gastrointestinal tract, is a prerequisite for early and adequate therapy. Multiplex PCR can identify a spectrum of the most common bacteria, viruses, fungi, and parasites associated with acute neuroinfectious, pneumonia, enterocolitis, and bacteremia/fungemia.

By multiplex PCR (FilmArray, bioMerieux, France) and standard microbiological methods were examined 111 cerebrospinal fluid samples, 76 positive blood cultures from the BactAlert automated system, 138 respiratory materials, and 24 stool samples. The clinical specimens were obtained from hospitalized patients with clinical and laboratory data for acute infections.

Our study of 349 patients showed that syndromic molecular biological testing with mPCR is a promising approach to increase pathogen detection rates and differentiate bacterial from viral and fungal etiology, despite the limited number of targets in the panels. Genes encoding antibiotic resistance directly from the obtained material, as well as associated infections, were also detected by mPCR. Of interest were some cases of children with respiratory co-infections from pertussis and rhinoviruses, SARS-CoV-2 and respiratory syncytial virus, as well as rhabdomyolysis induced by parainfluenza viruses, proven by mPCR. Analysis of 115 patients demonstrated that the newly introduced rapid methods reduced the hospital length of stay for patients in severe condition due to the timely change of antimicrobial treatment (from 34 days to 25.5 days); improved the outcome of the disease and significantly declined direct and indirect treatment costs (approximately BGN 12 500 per patient).

Our research confirms that the method can be used for timely and accurate determination of the etiological agent directly from the clinical specimen and is also suitable for detecting microorganisms as empiric antimicrobial therapy has already started. Early etiological diagnosis ensures timely initiation of targeted, instead of empiric treatment and contributes to economic cost efficiency.

Keywords: multiplex PCR, syndromic testing, acute infections

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MM3

HISTORICAL REVIEW OF TUBERCULOSIS VACCINES IN BULGARIA: COMPLETE GENOME SEQUENCE, GENOME STABILITY AND PHYLOGENY OF THE VACCINE STRAIN MYCOBACTERIUM BOVIS BCG SL222 SOFIA

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Tuberculosis vaccines are vaccinations intended for the prevention of tuberculosis. Immunotherapy as a defence against tuberculosis was first proposed in 1890 by Robert Koch. In 1906, Dr. Stamen Grigorov, previously known for his discovery of Lactobacillus bulgaricus, published a paper describing vaccination for prevention and treatment of tuberculosis applying filtrates of dead M. tuberculosis cells cultured with saprophytes. He continued this research and worked as a doctor throughout the rest of his life in the local hospital of his hometown Trun.

Mycobacterium bovis bacillus Calmette–Guérin (BCG) is the only live attenuated vaccine available against tuberculosis. The first BCG vaccination was done 100 years ago, in 1921. The BCG vaccine strains used worldwide represent a family of daughter sub-strains with distinct genotypic characteristics. BCG SL222 Sofia is a seed lot sub-strain descending from the Russian BCG-I (seed lot 374a) strain and has been used for vaccine production in Bulgaria since 1972. In this lecture we report the assembled circular genome sequence of Mycobacterium bovis BCG SL222 Sofia and phylogeny analysis with the most closely related BCG sub-strains. The full circular genome of BCG SL222 Sofia had a length of 4,370,706 bp with an average GC content of 65.60%. After 50 years of in vitro evolution in a freeze-dried condition, we identified four SNP mutations as compared to the reference BCG-I (Russia-368) sequence. BCG vaccination is of central importance for the TB elimination programs in many countries. Since 1991, almost 40 million vaccine doses of the BCG SL222 Sofia have been distributed annually through the United Nations Children's Fund (UNICEF) and the Pan American Health Organization (PAHO) to approximately 120 countries. The availability of the complete reference genome sequence for M. bovis BCG SL222 Sofia, a WHO reference reagent for the Russian BCG-I sub-strain, will facilitate the identity assurance of the genomic stability, will contribute to more consistent manufacturing, and has an important value in standardization and differentiation of sub-strains used in vaccine production. We propose to rename the sub-strain BCG SL222 Sofia to BCG-Sofia for practical and common use.

Keywords: BCG vaccine; BCG sub-strains; BCG-I; BCG SL222 Sofia; complete genome; phylogeny

Acknowledgements: This work was supported by the Bulgarian National Science Fund (BNSF) (grant DN 13/4-15.12.2017) and the European Fund for Regional Development through Operational Program Science and Education for Smart Growth 2014–2020 (grant BG05M2OP001-1.002-0001-C04).

MM4

ANTIMICROBIAL STEWARDSHIPS – PARALLELS

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Antimicrobial stewardship (AS) is a consensus multidisciplinary program, governmentally supported, dedicated to improve antimicrobial therapy of patients and to limit the development and spread of Antimicrobial resistance (AR). The aim of this work is to present the basic principles of AS, to describe the activities in Bulgaria and to make parallels with other countries.

Both literature review and data for Bulgaria was collected and analyzed. A comparison with activities in other countries was performed.

AS aims to deliver the most appropriate antibiotic/regimen to the patient, and to stop the further increase of AR. The basic principles, which should be followed by each physician are: - antibiotics (AB) should be prescribed for a proven bacterial infection, - specimen for microbiology investigation should be taken before, - clinical diagnosis and control of the focus of infection should be performed, - in severe cases empirical therapy should be prescribed, - upon receiving microbiology result/ after 48 or 72 hours, the therapy should be targeted to the most active narrow spectrum antibiotic, - after stabilization of the patient, a change of I.V. to P.O. route should be applied. In hospitals AS teams, consisting of physician - ID specialist, microbiologist and pharmacist are created. Two strategies may be executed: preliminary authorization, demanding the categorization of AB in three levels of prescription; or audit followed by a discussion with the prescribers. Considering rapid point of care tests is important.

In Bulgaria the first National program dates since 2001 and was started by the creation of Reference Laboratory on AR at the National Center for Infectious and Parasitic Diseases and by the introduction of BulSTAR surveillance program of AR. Nowadays in hospitals AS programs are required for their accreditation. A comprehensive National plan for AS was developed by multidisciplinary group in 2019, which still waits for acceptance by the Ministry of Health. At the same time countries like Australia, USA, all EU countries and many others have developed AS programs and already report the achieved positive results.

The successful AR surveillance, instituted more than 20 years ago, however is not sufficient for AS. Guidelines for rational use of AB, working organization in hospitals and ambulatory, education for healthcare workers and society, behavioral change for prescribers, collaboration with the Infection control and strict state participation are urgently indispensable.

The delay in establishment of working Bulgarian AS program is quite disturbing and requires active support from the Healthcare organizations and the Government.

Keywords: rational antibiotic policy; containment of antimicrobial resistance; Bulgaria

MM5

TARGETING GLMS AND FMN RIBOSWITCHES WITH ANTISENSE OLIGONUCLEOTIDES FOR ANTIBACTERIAL DRUG DEVELOPMENT

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Antibacterial resistance has become a significant issue worldwide due to the overuse and misuse of current antibiotics. Therefore, we must produce new antibiotics using novel, more efficient approaches. Such novel approaches must be more accurate, much faster, and economical than the classical ones based on small molecules. Here we present two papers recently published in ACS Synthetic Biology (Engineering Antisense Oligonucleotides as Antibacterial Agents That Target FMN Riboswitches and Inhibit the Growth of Staphylococcus aureus, Listeria monocytogenes, and Escherichia coli, Martina Traykovska Robert Penchovsky, ACS Synth. Biol. 2022, 11, 5, 1845–1855 https://pubs.acs.org/doi/10.1021/acssynbio.2c00013 and Targeting glmS Ribozyme with Chimeric Antisense Oligonucleotides for Antibacterial Drug Development - Martina Traykovska, Katya B. Popova and Robert Penchovsky, ACS Synthetic Biology, 2021, Q1, IF: 5,5, p. 3167–3176, ISSN: 2161-5063, DOI: https://pubs.acs.org/doi/10.1021/acssynbio.1c00443). This research developed a general approach for the precision design of antisense oligonucleotides that inhibit the growth of several human pathogenic bacteria. The approach is swift and accurate, giving us the tools to develop new antibacterial drug canditaes rapidly.

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MM6

GENOMIC CHARACTERIZATION OF UNUSUAL CASE OF FACIAL NECROTIZING FASCIITIS, CAUSED BY A NOVEL METHICILIN-SENSITIVE *STAPHYLOCOCCUS AUREUS* SEQUENCE TYPE (CC398-T1451)

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Facial necrotizing fasciitis (NF) due to *Staphylococcus aureus* (SA*)* is a relatively rare, but devastating and potentially lethal infection. The pathogenesis and progression of NF are a function of the complex interplay between the abundant virulence arsenal of SA and the host immune responses. Whole genome sequencing (WGS) has been established as the ultimate tool for studying virulence and resistance determinants in various pathogens. Herein we present a case of severe NF in a patient without any predisposing risk factors. A 36-year-old male was admitted at the Maxillofacial surgery clinic with a rapidly progressing clinical presentation and necrotizing lesions indicating NF. *S.aureus* was isolated from necrotic tissues (NF1) and blood cultures (NF2). Identification and antimicrobial susceptibility testing (AST) was performed by MALDI Biotyper® (Bruker) and BD Phoenix™, respectively. WGS was performed with Illumina DNA Library Prep and MiSeq v3 (600 cycles). Bioinformatic analysis (e.g, MLST, virulome, resistome) was conducted with a customized rMAP pipeline. The most closely related SA genomes (by ANI score) were downloaded from PATRIC and NCBI databases and phylogeny was inferred by the cano-wgMLST_BacCompare and the CSI Phylogeny.

The patient presented with a pronounced painful infiltrate localized in the left facial side, under his lower eyelid, that progressed later to necrotic lesions spreading across the left buccal mucosa and the soft tissues of the right shank and the shoulder. Multiple excisions and debridement of the affected tissues were performed. Both SA isolates were methicillin-sensitive (MSSA) with inducible MLS_B resistance phenotype. The assemled draft genomes were identical revealing *erm(T)* as the sole acquired resistance gene consistent with the AST findings. A total of 64 virulence-associated genes encoding 26 virulence determinants were detected despite the absence of the classical Panton-Valentine, enterotoxins and the Toxic Shock Syndrome toxins. Numerous toxins (α,β,γ-hemolysins and exotoxin), adhesins (autolysin, fibronectin binding proteins, *clfA*-clumping factor A etc.), immune evasion factors (CHIPS, SCIN etc), enzymes (Ser V8 protease, lipase, hyaluronidase etc.) and Type VII secretion system were identified. It was only recently elucidated that staphylococcal adhesins *atl* (autolysin), *fnbAB* (fibronectin-binding), *tet38* (tetracycline efflux) and *clfA* are involved in a unique process of bacterial internalization into the myoblasts followed by increased expression of intracellular cytotoxic effector molecules (RNAIII and psmα), underscoring their contribution to the pathogenesis of NF. The phylogenetic analysis revealed that NF1 and NF2 belong to a novel sequence type within the international clonal complex CC398, spa-type t1451 clustering together with strains from France, Germany and Japan. Successful antimicrobial therapy was achieved with vancomycin, cefazolin and clindamycin. The patient was discharged with improvement and recommendations for subsequent surgical reconstructive treatment. The occurrence of necrotizing infections due to *S.aureus* in patients without predisposing factors is alarming. MSSA CC398 is a hypervirulent pandemic clone associated with livestock but also with domestic animals, that represents a significant risk factor for human skin infections and NF.

Keywords: Necrotizing fasciitis, *Staphylococcus aureus, WGS, virulence*

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MM7

CHARACTERIZATION OF CEFIDEROCOL RESISTANCE IN MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA BY WHOLE GENOME SEQUENCING – PRELIMINARY RESULTS

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Infections caused by Multi-drug resistant *Pseudomonas aeruginosa* (MDR-PA) pose significant challenge for modern medicine severely limiting the therapeutic options. Novel beta-lactams and inhibitor combinantions like ceftolozan-tazobactam (CTL/TAZ), ceftazidime-avibactam (CAZ/AVI), imipenem-relebactam(IMI/REL), meropenem-vaborbctam (MER-VAB) are being introduced and show promising results although resistance is emerging. Cefiderocol (CDR) is a new generation siderophore cephalosporin having an exceptional activity against MDR bacteria. In the present work we report the first preliminary in-vitro results indicating the presence of CDR-resistant *P. aeruginosa* in Bulgaria and discuss the possible resistance mechanisms revealed by Whole-genome sequencing (WGS). A set of 85 clinical MDR-PA isolates from the collection of the National Reference Laboratory for Control and Monitoring of AMR were included in the study. Antimicrobial susceptibility (AST) was tested by either MICRINAUT-S (Bruker) or Liofichem MIC strips except for cefiderocol which was tested by disk diffusion (DDM) and data were interpreted according to the EUCAST. Expression profiling of genes known to confer cefiderocol resistance (*ampC, ampD* and *mexAB-OprM* efflux system) was performed with RT-qPCR. Whole genome sequencing was performed with Illumina DNA Library Prep and MiSeq v3 (600 cycles). Genomes were assembled and an extensive bioinformatic analysis was conducted with a customized rMAP pipeline. Mutations associated with cefiderocol resistance in *mexR, ampC* and its regulator *ampD* were studied with Geneious Prime package. A total of eight isolates (9.4%) were identified as non-susceptible to CDR and all of them were co-resistant to carbapenems including doripenem. Two were carbapenemase-producers with either Verona Integron-encoded MBL (VIM) or Imipenemase (IMP) and three were class A β-lactamase - producers (two PER and one VEB types). Acquired beta-lactamases, in particular MBLs and PER/VEB types are being discussed as having a significant role in cefiderocol non-susceptibility. In, addition seven isolates were resistant to both CTL-TAZ, CAZ-AVI and MER-VAB. Interestingly, six CDR-R isolates remained susceptible to imipenem-relebactam and colistin. Expression analysis showed overproduction of *ampC* in only one isolate, whereas overproduction of *mexAB-OprM* was not registered. All four isolates subjected to WGS had the *ampD* G148A substitution, considered a potential contributor to moderate CDR-resistance. Other previously reported mutations (e.g *ampC* E247K, *mexR* L57D, *mexR* A66V, *ampD* G116D) were not found in the present set. Four different sequence types were identified ST235 (n=1), ST621 (n=1), ST175 (n=1) and one ST111-like (n=1).

The rapid emergence of resistance against last-line drugs is raising concerns about public health. The precise resistance mechanisms to cefiderocol in MDR-PA still remain an enigma, but our data suggest an involvement of acquired beta-lactamases in addition to altered AmpC regulation. Therefore, efforts should be made to develop new strategies for control and monitoring of antibiotic resistance, as well as to implement new policies in the field of antibiotic consumption.

Keywords: cefiderocol, *Pseudomonas aeruginosa, WGS, resistance*

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MM8

CLOSTRIDIUM DIFFICILE INFECTION AMONG COVID-19

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Coronavirus disease 2019 (COVID-19) was first discovered in China in December 2019 and was declared a pandemic by the World Health Organization (WHO) on March 11, 2020. Despite the viral genesis of the disease, most clinicians began at different stages the use of oral or intravenous antibiotics. One of the main problems associated with long-term antibiotic abuse is *Clostridium difficile* infection (CDI), which leads to high mortality, especially among adult hospitalized patients. We studied 24 patients from three hospitals, with a mean age of 58 years (20-92 years) for the period March-July 2021, suffered from COVID-19 (proven by RT-PCR) and subsequently diagnosed with CDI (demonstrated by rapid toxin test and culture identification by enzyme-linked immunosorbent assay proving toxins). The 24 patients studied received their first symptoms of CDI on average 12.6 days after discharge from the COVID-19 units. Symptoms are diarrhea syndrome (100%), abdominal pain (83.3%), fever (75%). Some patients have leukocytosis (68.3%), less often with hypokalemia. Most patients have a premorbid history - leading are metabolic syndrome, diabetes, hypertension. 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 randomly selected 60 patients, banana peel fiber (Banatrol®) was used along with the classic diet. In these patients, the diarrhea syndrome lasted on average 2.6 days less and we observed only 4 patients with relapse. COVID-19 treatment continues to be subject to aggressive antibiotic therapy, in many cases unnecessary. 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.

Key words: *Clostridium difficile* infection, COVID-19, diarrhea, antibiotic.

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MM9

NOVEL COMPOSITES BASED ON ACTIVATED CARBON AND CU, AG, AND MG AS ANTIBACTERIAL AGENTS

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Nowadays there is constant emergence of agents threatening human health, such as air pollution, terroristic attacks with chemical and biological agents, COVID-19 pandemic. There is a need to develop such facilities and materials to cope with this wide range of hazards in the air of public buildings, hospitals and even our homes. Activated carbon composites (ACC), containing metal nanoparticles are promising developments in this direction.
As methods of structural and morphological analysis SEM, elemental analysis and X-ray diffraction were performed. Antimicrobial activity of the composites was evaluated by using *Escherichia coli* K-12 (ATCC 25922) as test microorganisms and ASTM Standard Test Method E 2149–10. To working bacterial dilution, composites (with particle size of 0.4 mm) containing Cu, Ag, Mg, were added. Two controls were prepared as well- bacterial suspension /bacteria only/ and bacterial suspension with activated carbon. Samples were taken at 2 min, 1h, 24h, and 48h. The bacterial concentration in the solutions was evaluated by performing serial dilutions and standard plate counting techniques. The number of colonies in the Petri dish after incubation (CFU/ml) was measured.
SEM pictures of resulting materials show Distribution of metal nanoparticles on activated carbon matrix. They have BET surface area of 1300 m2/g, contain 73.42 % C, 0.52 % H, 0.24 % N, 2.78 % S, and show high graphitization degree, obtained by XRD.
The results indicate that the antibacterial activity depends on the contact time and metal content. Best result was observed when Cu ACC was used with 100% reduction of microbial count at first minutes. Ag ACC showed about 5 log reduction of bacteria 1h later. On the first and the second day there were no bacteria in the solutions where ACC and activated carbon only were added.
Preparation of metal nanoparticles incorporated activated carbon composites provides a novel material with antibacterial activity for further development and optional application in hygiene devices and individual masks.

Keywords: novel antibacterial agent, metal nanoparticles, activated carbon

MM10

PRIMARY MULTIDRUG RESISTANCE AND MAJOR CLUSTERS OF THE EPIDEMIOLOGICALLY SIGNIFICANT *MYCOBACTERIUM TUBERCULOSIS* BEIJING GENOTYPE IN NORTHWEST RUSSIA

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Tuberculosis (TB) incidence is decreasing in Russia but the country remains in the top-30 list of countries with high level of multidrug resistant TB (MDR-TB) and HIV-associated TB. *Mycobacterium tuberculosis* lacks horizontal gene transfer and therefore its population structure is clonal and hierarchical. It includes large phylogenetic lineages, smaller sublineages and genotypes and compact clonal clusters of genetically related strains. Certain genetic variants are of greater clinical and/or epidemiological significance than others that is manifested as high virulence, transmissibility, association with MDR. The Beijing genotype is the most studied genotype of *M. tuberculosis*. In Russia, the Beijing strains are the most prevalent on the whole and play the major role in the spread of MDR-TB. The Beijing genotype is subdivided into modern and ancient/ancestral sublineages. Most of the Russian Beijing population consists of the modern clusters (including epidemic Beijing B0/W148 and Central Asian/Russian) and less prevalent but MDR Beijing 1071-32 and 14717-15-clusters of the ancient sublineage. The aim of this study was a molecular characterization of the *M. tuberculosis* Beijing strains from newly-diagnosed TB patients in Northwest Russia. We studied 631 *M. tuberculosis* strains isolated in 2014-2019. Bacterial culture and drug susceptibility testing were carried out by standard methods. The Beijing genotype, its ancient and modern sublineages, and main epidemic and endemic clusters were determined by the PCR and real-time PCR based methods developed in our laboratory. 24-loci VNTR typing was performed as described and profiles were compared to the MIRU-VNTRplus tool (https://www.miru-vntrplus.org). More than half of strains belonged to the Beijing genotype (55.6%; 351/631), of which 50.4% (177/351) were MDR. Most of strains belonged to the modern Beijing sublineage (341 isolates), while 10 to the ancient one. The strains of the modern sublineage fall within Central Asian/Russian (217, of which 38.2% were MDR) and B0/W148 (92, of which 84.8% were MDR) clusters. MIRU-VNTR typing of 288 Beijing strains revealed 82 profile variants. Twenty-four clusters (2-75 isolates) included 79.9% strains. The largest clusters within Central Asian/Russian strains were 94-32 (n=75), 1065-32 (n=17), and 95-32 (n=12), within B0/W148 strains - 100-32 (n=59) and 4737-32 (n=5). MDR was more often detected in clusters 1065-32 (88.2%), 100-32 (83.1%), and 4737-32 (100%). Interestingly, 25 modern Beijing isolates were not included in the two above clusters and among them, the modern Beijing cluster 9391-32 (n=9) was represented only by drug-susceptible strains. Eight MDR strains of the ancient Beijing sublineage belonged to the 1071-32 cluster.

In conclusion, *M. tuberculosis* population in North-West Russia is dominated by the Beijing genotype (55.6%). Half the Beijing strains is MDR. The Beijing Central Asian/Russian cluster was the largest (61.8%), followed by B0/W148 (26.2%). The B0/W148 cluster included predominantly (84.8%) MDR strains. Thus, increasing proportion of primary MDR-TB is due to active transmission of Beijing strains, mostly MDR B0/W148 strains also known to rapidly acquire resistance to new anti-TB drugs. This poses a serious problem of the TB control and treatment of such patients, since MDR-TB treatment is known to be very expensive.

Keywords: *Mycobacterium tuberculosis*, primary multidrug resistance, Beijing genotype, Central-Asian/Russian cluster; B0/W148 cluster

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MM11

DIRECT IDENTIFICATION OF *YERSINIA PSEUDOTUBERCULOSIS* IN RAW MILK BY DIGITAL DROPLET PCR AND LOOP MEDIATED ISOTHERMAL AMPLIFICATION

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According to the European Food Safety Authority yersiniosis is the third most frequently reported zoonosis in the European Union (EU). Out of 5,668 human cases registered in 2020, *Yersinia pseudotuberculosis* was the causative agent in 94 (6.17%). As a psychrophilic pathogen, *Y. pseudotuberculosis* is responsible for the most commonly foodborne infections with symptoms of enterocolitis or mesenteric lymphadenitis (pseudoappendicitis) in children. The trend of its isolation from raw sheep, goat and cow milk is particularly alarming. There is currently no harmonized monitoring plan for detection of *Yersinia*in food or animals in the EU. PCR-based methods offer the advantages of speed and higher sensitivity compared to culture-based methods for detection of this pathogen. Therefore, intensive research is currently underway for creation of new molecular biology methods for rapid and cost-effective diagnosis. The aim of the current study was to develop fast and robust identification methods for direct detection of *Y. pseudotuberculosis* in goat milk using digital droplet PCR (ddPCR) or loop mediated isothermal amplification (LAMP).

Samples of *Yersinia*-free raw goat milk were artificially contaminated with logarithmically decreasing concentrations of *Y. pseudotuberculosis*. The DNA extracted thereof was used for optimization of the ddPCR and LAMP protocols. The *Y. pseudotuberculosis* primer's set for the LAMP reaction was chosen from published data [1,2] and proven for specificity on a panel of foodborne bacterial pathogens (*Yersinia* spp., *Campylobacter* spp., *Salmonella* spp. and *E. coli*). Four different kits for LAMP assays were compared for sensitivity. The ddPCR protocol was developed using primers for detection of the gene *ail* in *Y. pseudotuberculosis* and a probe labelled with the fluorophore 6-FAM and the quencher BHQ [3].

The chosen LAMP primer's set exhibited high selectivity for *Y. pseudotuberculosis*. The optimization of the protocols led to promising results concerning the detection limit of the method. The highest sensitivity was achieved with the EURx Blue-LAMP – below 10 copies/reaction and between 10^1 and 10^2 DNA copies/mL which is comparable to the sensitivity of the ddPCR method. The ddPCR protocol should be applied for direct quantitative detection of the targeted pathogen.

In conclusion, the presented ddPCR and LAMP protocols are characterized by high sensitivity and selectivity. They can be used for fast and reliable detection of the pathogen in raw milk.

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MM12

ANTIMICROBIAL EFFECT OF SILOXANE COATINGS WITH ANTIOXIDANTS

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Hospital-acquired infections are known for a long time as some of the major problems relating hospitalization nowadays. An example for this kind of infections is due to the bacterial biofilm formation on catheters. This type of complications are a serious threat for a patient's life and that's the reason that scientists are focused on finding a solution. In addition, this is the main aim in the project, which is a collaboration between Sofia University "St. Kliment Ohridski" and University of Chemical Technology and Metallurgy – testing different surfaces, synthesized from polymers and nanoparticles, against bacterial adhesion and biofilm formation. Nanocomposites represent an organic or inorganic carrier saturated with nanoparticles of organic or inorganic origin.

The bacteria, used for the experiments, are the Gram-negative *Escherichia coli* ATCC 25922 and the Gram-positive *Staphylococcus aureus* ATCC 25923. We measure the growth, vitality and count of the microorganisms for every zero, 24th and 48th hour. The obtained results vary due to the nature of the surfaces and type of bacteria. With the second type of coating, the best antibacterial effect is the fourth variant, in which the two tested bacteria formed a relatively weakly attached biofilm at the 24th hour. At the 48th hour, the *S. aureus* biofilm had the weakest adhesion, in which the amount of attached cells was reduced by 2 orders of magnitude. At the 24th and 48th hours, the third type of coating has a stimulating effect on biofilm formation by both bacteria, therefore this coating is not suitable for medical purposes. With the fourth type of coating, the reported amounts of *E. coli* and *S. aureus* in the biofilms of all 6 tested variants are close to the control ones. Only the fourth variant at 24 hours and the fifth variant at 48 hours had a weak effect on *S. aureus*. The fifth type of coating has no effect on the tested bacteria for the first 24 hours, but during the second, inhibition of the first variant can be reported only on *E. coli*, but not on *S. aureus*. The sixth type has a weak inhibitory effect on the two tested bacteria with its last variant. This effect was enhanced on *E. coli* at 48 hours, but, unfortunately, the same variant had no effect on *S. aureus*. The second variant of the sixth type of coatings has a weak inhibitory effect at the 24th hour, which increases at the 48th hour, and the inhibition is about 2 and more orders of magnitude for both bacteria. The morphological characteristics of bacteria are of particular importance for the effect of antioxidants on them. Rinsing of the coatings is recommended to assess the strength of the biofilm formed.

Keywords: antibacterial effect, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923

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MM13

PCR ANALYSIS FOR *MYCOBACTERIUM TUBERCULOSIS* COMPLEX SHOWS NEGATIVE RESULTS IN SARCOID SAMPLES

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Sarcoidosis is a systemic granulomatous inflammatory disorder that affects multiple organs. Despite numerous studies, the aetiology of sarcoidosis is still a medical mystery. Several microorganisms are suspected as etiological agents causing sarcoidosis. *Mycobacterium tuberculosis* is one of the most investigated bacteria. Due to the histological and clinical similarities between sarcoidosis and tuberculosis, the role of mycobacteria has been constantly investigated as an aetiological agent for sarcoidosis. Several studies using molecular techniques find a possible relationship between *M. tuberculosis* or nontuberculous mycobacteria with sarcoidosis aetiology, while others don't [1,2]. Goal of our study was to investigate the possible link between sarcoidosis aetiology and *Mycobacterium tuberculosis* complex. We investigated blood, biopsy and bronchoalveolar lavage (BAL) samples of sarcoidosis patients with conventional PCR analysis, targeting the insertion element IS6110.

Clinical samples from 28 patients suspected with sarcoidosis were obtained from University Multiprofile Hospital for Active Treatment of Pulmonary Diseases "St. Sofia". Total DNA was isolated from each type of sample using lysis buffer containing NaCl (500mM), EDTA (50mM), Tris-HCl (50mM), SDS (4%) and 0,2 mg/ml proteinase K (2mg/ml, Invitrogen) and beating with zirconium beads (0.3 μm and 0.5 μm) on BeadBug Microtube homogenizer. After the lysis proteins were precipitated with 10 M ammonium acetate. DNA from the supernatant was precipitated with isopropanol. DNA pellet was washed with 70% ethanol and finally dissolved in 50-100 μl of sterile water for PCR (Canvax, Spain). For analysis, we included samples from histologically confirmed sarcoidosis cases n=11. The first group of samples included paired lung biopsy material, and blood from 5 patients. The second group of samples were collected from 6 patients with sarcoidosis and included lung biopsy, blood and BAL for each patient. For *M. tuberculosis* complex (MTC) detection we used primers for the repetitive insertion element IS6110 with an amplicon size of the PCR product - 113 bp. As a positive control, we used DNA isolated from *M. tuberculosis* culture. Pure water was used as a negative control. PCR products were visualized by 2 % agarose gel electrophoresis.

In this study, the IS6110 repetitive DNA element of MTC was not detected in any of the samples from the patients with sarcoidosis.

Mycobacterial DNA was not identified in any sample type – blood, lung biopsy or BAL from 11 confirmed sarcoidosis cases. For precise conclusions, more sarcoid samples should be studies.

Keywords: sarcoidosis, aetiology, *Mycobacterium tuberculosis* complex

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MM14

ANTIMICROBIAL EFFECT OF MAGNETIC NANOCERAMICS

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Nanoparticles, with sizes below 100 nm, have been the subject of many different types of research in recent years, as well as those looking for a new approach to dealing with different types of bacteria, viruses and parasites. Magnetic nanoparticles are also of interest for this kind of research and many of them show good results. Magnetic nanoparticles are an ideal drug carrier and can be used for antitumor therapy due to their easy control, targeting, and removal from the human body. For them as drug carriers, their antimicrobial effect is extremely important. In the report, the antimicrobial effect of magnetic nanocomposites with various metals included against standard microorganisms, representatives of Gracilicutes and Firmacutes bacteria were investigated.

The bacteria, used for the experiments, are the Gram-negative *Escherichia coli* ATCC 25922 and the Gram-positive *Staphylococcus aureus* ATCC 25923. In the study of pure nano-sized copper oxide-tenorite, designated as nCuO, a stronger growth inhibitory effect was reported on *E. coli* than on *S. aureus* at a concentration of 0.62 mg/ml. According to the obtained results, it can be noted that the most active nanocomposite against *E. coli* in the first 24 hours is ZnOFe. The MBC of ZnOFe is 0.62 mg/ml. The MIC of ZnOFe is 0.3 mg/ml. There is a pronounced antibacterial effect of the ZnOFe nanocomposite against *S. aureus* with MBC – 1.25 mg/ml and MIC – 0.62 mg/ml and time-dependent suppression. The CuZnFeO ternary complex is also active against *E. coli,* with an MBC of 2.5 mg/ml and an MIC of 1.25 mg/ml in the 24th hour. With CuZnFeO, a time-dependent bactericidal effect on *E. coli* was also observed at a concentration of 1.25 mg/ml at 72 hours. For CuFeO at a concentration of 2.5 mg/ml, a time-dependent antibacterial effect against *E. coli* 25922 is manifested, and at the 24th hour the MBC is higher, probably 5 mg/ml. For *E. coli* at 72 hours the MBC was 1.25 mg/ml. For *S. aureus*, CuFeO has a bactericidal effect at a concentration of 2.5 mg/ml per 24 hours, and the minimum inhibitory effect is 1.25 mg/ml. The CuZnFeO complex is also active against *S. aureus* with an MBC of 2.5 mg/ml for 24 hours. Prolonged exposure leads to a stronger effect (time-dependent effect) with ZnOFe and CuZnFeO. There is a stronger effect of nanocomposites on *E. coli* than on *S. aureus*.

Keywords – magnetic nanoceramics, copper oxide, copper ferrite, copper-zinc ferrite, zinc ferite nanoparticles

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MM15

EFFECTS OF AUTOCHTHONOUS (WILD) *LACTOCASEIBACILLUS PARACASEI* STRAINS ON ALP ACTIVITY IN NON-DIFFERENTIATED AND DIFFERENTIATED HUMAN COLORECTAL ADENOCARCINOMA HT 29 CELLS

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Lactobacillus species are non-pathogenic lactic acid-producing bacteria (LAB), which reveal probiotic properties and are technologically suitable for industrial processes. The alkaline phosphatase secreted into the lumen by enterocytes plays an essential role in fat and phosphate metabolism. It inactivates bacterial pathogens by producing antimicrobial compounds and promotes bacterial colonization of the intestine with commensal organisms. The HT29 cell line is a valuable model for studying the biology of human colon cancers, food digestion, and bioavailability. The current study aimed to determine if the supernatants achieved from potentially active autochthonous *Lactobacillus paracasei* strains isolated from native ecological niches in Bulgaria possess any probiotic potential in non-differentiated and differentiated human colon adenocarcinoma cell line HT29. The wild strains of LAB were isolated from mountain anthills of red wood ants (Formica rufa L.) by wooden sticks placed into anthills located at Sinite Kamani National Park, Bulgaria. The HT 29 cells (ATCC, HTB-38™) were cultured on 24-well plates in basal media (BM: DMEM, 10% FBS, and an antibiotic), in humidified 37^{o}C and 5% CO2 conditions. Twenty-four hours later, they were treated with supernatants (SN) derived from four Lactobacillus paracasei as follows: C8, P4, M 2.1, and C 15. The strains were cultured in MRS for 24 hours, centrifuged, and the SN filtered true 20 µm syringe filter. The half of each SN was neutralized up to pH=7. The non-differentiated and differentiated (the differentiation protocol as described by [7]) cells were treated for 24h with 10 % SN dilutions in BM (native and neutralized for each strain). Additionally, the cells from both groups were cultured in 10 % MRS for 24h to allow for its effect. After the treatment period, the ALP activity in experimental culturing media was estimated colorimetrically by BS-120 Chemistry Analyzer (MINDRAY, China) using ALP reagent (Biolabo reagents, Franse). The ALP activity was also measured in SN from C8, P4, M 2.1, and C 15 themself. The 10% neutralized SN from *Lactobacillus paracasei* supplemented culturing media causes in differentiated HT29 cells a significantly ($p<0.001$) rising of ALP activity in all treatments with an average of 18% compared to its relative MRS control. Differentiated cells showed significantly ($p<0.001$) higher ALP activity than non-differentiated ones in all SN treatments. The most substantial effects were observed in the C8 (20%) and M 2.1 (21%) groups, where the ALP activity was significantly higher than in P4. In non-differentiated cells, the SN from P4 and C8 was detected as an insignificant suppression of ALP activity since C15, and M2.1 treatments had an adverse influence. Alkaline phosphatase activity was not detected in 10% treatment media with SN from C8, P4, M 2.1, C15 and MRS prior to the cell treatment.

In conclusion, the current preliminary study revealed that all isolated wild Lactobacillus species manifested probiotic properties by significantly enhancing the AP activity in differentiated HT29. We could speculate that C15 and M2.1 might exhibit anticancer effects in non-differentiated cells, but additional, more profound studies are necessary to confirm that statement.

Keywords: *Lactocaseibacillus paracasei*, probiotic effects, HT29

MM16

AUTOCHTHONOUS *LACTOCASEIBACILLUS PARACASEI* STRAINS ENHANCE GLUCOSE UPTAKE IN MATURE 3T3-L1 ADIPOCYTES

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Obesity has been cited as a significant risk factor for morbidity and mortality worldwide. Abnormal adipocyte growth is often accompanied by gut dysbiosis, both associated with metabolic disorders, including insulin resistance, diabetes and cardiovascular diseases [1]. Numerous food and chemical additives were found to reduce obesity and obesity-related disorders, but many of them with significant adverse effects. Nowadays, attention has been focused on weight lowering and insulin sensation potential of numerous probiotic cultures with proven benefits to the host health [2]. Among them, *Lactobacillus species* and their derivated reveal promising advantages. Their effects are often associated with improved glucose tolerance, type 2 diabetes and obesity in animals and humans [3,4]. However, positive results were prevalent when bacteria, their lysates or supernatants were applied as a prevention rather than therapy of obesity [5,6]. Therefore, the current study aimed to investigate the impact of *Lactocaseibacillus paracasei* (LP) supernatants derived from autochthonous strains inhabiting native ecological niches in Bulgaria on adipogenesis, lipolysis rate and glucose uptake in already mature 3T3-L1 adipocytes.

The LP strains: M2.1, C8, C15 and P4 were isolated from native anthills of red wood ants (Formica rufa L.) located at Sinite Kamani National Park, Bulgaria by wooden sticks and cultured in a de Man, Rogosa, and Sharp media (MRS) for 24h. Then the microorganisms were separated from the MRS broth by centrifugation, and the supernatants were filtered, normalized up to 7 pH and stored at -20°C until use. Meanwhile, confluent 3T3-L1 (ATCC), grown in 24-well plates in basal media with 4.5 g/L glucose concentration (BM), were subjected to adipogenic induction for 48h, followed by seven days of maturation in BM, supplemented with 10μM insulin. Five groups (n=6) were formed at this stage: four experimental - M2.1, C8, C15, and P4, treated with 10% supernatants in BM of the respective LP strain and one control – treated with 10% MRS (MRS_C). Forty-eight hours after treatment, cell viability, intracellular lipids accumulation, glucose and glycerol concentration were measured.

The obtained results indicated that all tested strains' supernatants of LP improved glucose utilization by more than 22% compared to the MRS_C and did not affect the cell viability of mature adipocytes after 48h of treatment. However, the modulation of lipid accumulation and lipolysis rate heavily depends on the used strain type. The supernatants from C8 and C15 decreased intracellular lipid accumulation without significant lipolysis rate changes. Contrary, the M2.1 supernatants increased the intracellular lipids deposition by 18% and reduced lipolysis by 22% compared to MRS_C. In P4 group glucose uptake was enhanced to the greatest extent (40%), but no changes in the other studied parameters were found.

In conclusion, the current findings showed that all tested LP strains improved glucose utilization of the mature adipocytes, which *in vivo* could significantly benefit the health status of obese individuals. As previously demonstrated, this was achieved by specific metabolic pathways modulation of each LP strain. Further investigation is needed to clarify the exact intracellular mechanism of action.

Keywords: *Lactocaseibacillus paracasei*, glucose uptake, lipolysis, adipogenesis, 3T3-L1

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Veterinary Microbiology

VM1

ROLE OF PIG FARMS IN THE SPREADING OF ANTIMICROBIAL RESISTANCE

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The Bulgarian pig industry currently produces approximately 10% of the total meat produced in the country and in this process a wide range of pathogenic bacteria are causing infectious diseases. Therefore, the challenge of antimicrobial resistance (AMR) needs to be addressed not just in animals or in humans, but in all contexts of the One Health approach, and the choice of antimicrobials for treatment of pigs has to be recognized as a main factor driving the emergence and spread of bacterial resistance worldwide. Herewith, the prevalence of resistant *Escherichia coli* strains from swine feces and lagoons was evaluated according to ISO 16654:2001/Amd1:2017. The biochemical characterization and AMR were investigated by Phoenix M50 and disc diffusion method, according to CLSI and EUCAST. The suspected colonies from feces and lagoons were confirmed by 16S rDNA PCR and evaluated to form biofilms. Some isolates showed phenotypic resistance to ampicillin, trimethoprim, trimethoprim/sulfamethoxazole, amoxicillin, tetracycline, chloramphenicol, etc. Their pathogenicity was determined by conventional and multiplex PCR. We investigated the prevalence of antibiotic-resistance genes (ARG) to quinolones (*qnrA* and *qnrB*), aminoglycosides (*aac(3)-IV*), erythromycin (*ermB*) and β-lactam antibiotics (*amp*C, *blaSHV*, *blaCMY* and *blaTEM*) and proved the presence of *ampC* in 7, *blaSHV/blaCMY/blaTEM* in 1 and *ermB* in 1 isolate. All strains were negative for the virulence genes (ETEC - LT, STa and F4), EPEC (*eae*) and STEC/VTEC (*stx1* and *stx2all*). With the optimized ddPCR protocol, it was possible to detect the presence of pathogenic *Yersinia enterocolitica* after direct isolation of DNA from swine feces samples found negative for this pathogen by applying classical microbiological methods. The amount of *Y. enterocolitica* was 1.2×10^3 DNA copies per reaction which corresponds to 4×10^4 CFU/g feces.

In conclusion, there is evidence for the presence of AMR bacterial strains in swine feces and risk for dissemination of ARG in the surrounding area through wastewater and fertilizing of soil. The implemented ddPCR protocol should be applied for direct detection of viable but non-culturable pathogenic *Y. enterocolitica* in swine feces, wastewaters, or lagoons.

Keywords: pig farms, antimicrobial resistance, multiplex and ddPCR, *Escherichia coli, Yersinia enterocolitica*

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VM2

STUDIES ON HERPESVIRUS INFECTIONS IN RUMINANTS

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In order to limit the great economic losses that herpesvirus infections cause to animal husbandry and new ways of raising productive animals, it is necessary to develop and apply different methods - virological, serological and molecular-biological, through which a quick and accurate diagnosis can be made. This necessitates both a study on their distribution among domestic and wild animals, as well as the development of new, more sensitive methods for the proof and characterization of virus isolates. In order to establish the spread of herpesvirus infections in affected animals and to isolate and prove the causative agent, it is necessary to take into account the reactivation and emission of herpesviruses. After the latency period, herpesviruses reach a state of persistence and may become exalted under natural or induced stress. The reactivation and shedding are from the place of initial entry of the viruses into the body.

Through serological methods, virus neutralization and ELISA reactions, we conducted studies on the spread of infections caused by different herpes viruses. Using virological methods, we studied the sensitivity of different types of cell cultures to the development of isolated herpes viruses and their physicochemical characteristics. The characterization of different types of herpes viruses isolated from ruminants was carried out by molecular biological studies. For this purpose, we used different variants of the polymerase chain reaction (PCR), restriction genome analysis with different types of restriction enzymes to study the whole or a separate part of the virus genome, and sequence analysis of individual genes of the isolated herpesviruses.

In the serological studies by means of virus neutralization and ELISA reactions in the different species of animals, we proved the spread of the diseases from 21.3 to 65.2%. Using virological methods, we found that the most suitable for cultivation and for carrying out biochemical studies are the cell cultures of bovine, rabbit, and sheep origin. Through different PCR variants, we amplified the genes - gB, gC, gD, gE and TC of the bovine herpes virus 1 (BHV 1), and for the caprine herpes virus 1 (CHV 1) we amplified two parts of the gC gene, 414 bp and 527 bp in size . Using specific primers, we amplified gB and TK genes of bovine herpes virus 4 (BHV 4) with size 615 bp and 567 bp. When performing a nested variant of PCR to prove the TK gene, were obtained an amplification product with a size of 260 bp. When conducting PCR for the MCF virus, we obtained amplicons with a size of 422 bp, and after the nested reaction with a size of 238 bp. When conducting restriction enzyme genomic analysis of the entire BHV 1 genome, we found that the most suitable restriction enzymes (RE) were Hind III and Sca I, by which we successfully differentiated respiratory from genital and abortogenic isolates of the virus. Abortogenic herpesviruses cannot be clearly differentiated using RE Eco R I and Hpa I. By performing a restriction enzyme analysis of the gC gene of BHV 1, we found that the three types of BHV 1 could be successfully subtyped after using RE Ehe I. Bam HI RE successfully distinguished BHV 1 from swine herpesvirus 1 (SHV 1). For restriction endonuclease analysis of the complete genome of BHV 4, the most suitable REs were Hpa I, Bam HI, Eco R and Hind III, which detected fragments of polyrepetitive DNA (Pr DNA). By REA of the gC gene of CHV 1 by RE Hpa II and Msp I, we found that the Bulgarian CHV 1 isolates have a similar restriction enzyme profile to the American strain "McK" and differ from that of the Western European CHV 1 isolates "E/CH", " Spain 1" and "Spain 2". The different genes from the collection of BHV 1 (gC, gB, gE and gD), BHV 4 (gB and TK) and CHV 1 (gC) isolates were amplified with the specific primers. The obtained PCR products were sequenced and compared with the sequences of the corresponding genes of BHV 1, BHV 4 and CHV 1 deposited in the genome bank. Based on the nucleotide and amino acid sequences, we constructed phylogenetic trees of the investigated isolates and compared them with the reference strains of BHV 1, BHV 4 and CHV 1, thereby confirming the identity of the Bulgarian herpes viruses and their location.

As a result of the conducted studies, new data were obtained on the spread of herpes virus infections in our country and the herpes viruses isolated from animals were characterized in more detail.

VM3

ISOLATION AND IDENTIFICATION OF *FLAVOBACTERIUM PSYCHROPHILUM* FROM TROUT HATCHERY IN BULGARIA

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Herein, we describe the first isolation and whole genome sequencing of *Flavobacterium psychrophilum*, aetiological agent of bacterial coldwater disease (BCWD) and rainbow trout fry syndrome (RTFS), from infected Rainbow trout (*Oncorhynchus mykiss*) fry in Bulgaria. Fish samples from one and the same hatchery were accepted in the NRL for fish, mollusk and crustacean diseases of the National Diagnostics and Research Veterinary Medical Institute - Sofia in October 2021 (case1) and January 2022 (case 2). Mortality started when the fry reach a size of 10-15 g in the first case and 1-1.5 g in the second case. Bacterial isolates from the skin, kidney, liver and spleen of all diseased fish were yellow orange on Shieh agar, Gram-negative, filamentous bacilli that were oxidase and catalase positive, and produced a flexirubin-type pigment in 3% potassium hydroxide. Isolates identity was confirmed by *F. psychrophilum*-specific PCR, MALDI-TOF MS and whole genome sequencing. Isolates from both cases were confirmed as different from each other and representing novel MLST types by NGS. While antimicrobial resistance genes were absent a significant number of virulence factors were present – adhesins, siderophores, Type III secretion system, curly fimbriae etc. Phylogenomic SNP analysis revealed that Bulgarian *F. psychrophilum* strains clustered most closely with *F. psychrophilum* isolated from Rainbow trout in Finland. Findings illustrate the potential for *F. psychrophilum* to cause acute infections in Bulgarian rainbow trout hatcheries and highlight the need for future investigations on *F. psychrophilum* transmission.

Keywords: *Flavobacterium psychrophilum*, virulence, mortality, Rainbow trout fry, NGS

VM4

FIRST ISOLATION AND IDENTIFICATION OF *VIBRIO AESTUARIANUS* FROM THE BULGARIAN SEA AREA

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Vibrios are gram-negative marine bacteria widely distributed in estuarine and coastal waters and sediments. The genus comprises not only human pathogens (*Vibrio cholerae*, *V. vulnificus*, *V. haemolyticus*) but also species pathogenic for aquatic animals as *V. aestuarianus*. *V. aestuarianus* is able to survive in diverse marine environment, aquaculture farms, and marine invertebrates. Diseases caused by *V. aestuarianus* have posed threats to aquaculture facilities worldwide. In this study, *V. aestuarianus* was isolated and confirmed for the first time from Bulgarian Black Sea aquatoria. The risk of disease and mortality in oysters grown in Black Sea waters is outlined.

Keywords: *V. aestuarianus*, Bulgarian sea area, risk assessment

VM5

MICROBIOLOGICAL STUDIES ON THE PREVALENCE OF *STAPHYLOCOCCUS* SPP., INVOLVED IN THE ETIOLOGY OF MASTITIS IN CATTLE AND THEIR SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS IN THE REPUBLIC OF BULGARIA

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For the period June 2020-March 2022, a total of 8 dairy cattle farms were surveyed in terms of the prevalence of clinical and subclinical mastitis. Four of them were located in Northern Bulgaria (Targovishte-2, Shumen and Dobrich districts) and another four in Southern Bulgaria (Stara Zagora, Plovdiv and Haskovo districts). In these target farms, a rapid mastitis screening test was initially performed to detect the presence of subclinical mastitis or high somatic cell samples. A total of 312 samples of milk secretion were obtained from those who reacted with 3+ or 4+ milk quarters to which 34 samples of inflammatory exudate were added in cases of cows with clinical mastitis. During the microbiological investigation from the 346 samples, a microbial finding was found in 272 of them (79.1%). *Streptococcus* spp., were isolated from 151 samples, respectively, 55.5%. The second most common species were *Staphylococcus* spp., they were determined in 110 of tested samples or 40.4 % of them. In total, the Gram-positive cocci finding exceeded 95% of the microbial species. The remaining 11 (4.0%) isolates belonged to another 6 taxa. These included four strains identified by prior phenotypic identification as *Trueperella pyogenes*, 3 *Escherichia coli* isolates, and one strain each of *Pasteurella multocida*, *Nocardia asteroides*, *Klebsiella pneumoniae*, and *Acinetobacter* spp.

Staphylococci were also studied for the sensitivity to 11 chemotherapeutic agents. The highest percentage of resistance (50%) was determined to lincomycin, followed by the tetracycline (37.3%), and beta-lactams, ampicillin (24.5%), and oxacillin (24.5%). The prevalence of resistance to cefoxitin and caphalotin was 5.5% and 0.9% respectively. Also lower values of resistant strains were observed for the combination of trimethoprim/sulfonamides (7.3%), ciprofloxacin (1.8%), and rifampicin (0.9%). Resistance to gentamicin and amoxicillin/ clavulanic acid has not been established. Minimum inhibitory concentrations were determined for the studied chemotherapeutics, with the highest values of MIC_{90} 128 μg/mL and 2 μg/mL analyzed for tetracycline and lincomycin, and the lowest values of MIC_{90} 0.001 μg/mL for rifampicin and ciprofloxacin, respectively. The MIC_{90} 1.5 μg/mL analyzed for oxacillin and trimethoprim/sulfonamides, and 1.0 μg/mL for ampicillin, cephalotin and cefoxitin.

Keywords: mastitis, cattle, staphylococci, resistance

VM6

ON SOME BASIC HARACTERISTICS OF *STREPTOCOCCUS SUIS* STRAIN ISOLATED FROM INTENSIVE REARED PIGS IN BULGARIA

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Streptococcal infection is one of the problematic bacterial diseases in pigs raised under industrial conditions. It affects animals of all ages, but the most sensitive are suckling and growing pigs. The losses from streptococcus infection are significant and create a lot of worries for farmers. The causative agent *Streptococcus suis* is characterized by a large serotype diversity, based on antigens of the capsular polysaccharide. At this moment 35 serotypes are known, as serotype 2, which has been proven to be zooanthroponotic, has become especially relevant. This makes the infection very important for public health. This communication reflects the results of a study on the main microbiological characteristics of 58 streptococcal strains. They were isolated from the nasal mucosa of newborn pigs or from the soft birth canal of repair female animals (primates) or parturient sows. Streptococcal isolates were obtained by plating on blood agar with 5% sheep blood and after that were cultured under microaerophilic conditions. The same strain was biochemically identified by conventional microbiological techniques. They also were studied in terms of their cultural features and morphological characteristics after Gram staining.

It was found that all isolated strains have pronounced β-hemolytic activity, but they did not produce catalase and oxidase. It became clear that 97% of them hydrolyzed esculin in the presence of bile salts. In the same time 88%of strains fermented glucose with lactic acid production without gas. All isolated strains also degraded trehalose. Regarding the mannitol, most of the strains (92%) also turned out to be positive. It turned out that majority of strains give scanty growth in NaCl medium. Latex agglutination determined their group affiliation as belonging to group D according to Lancefield. Attempts to prove the presence of such serotype 2 among isolates studies have shown that only 4 strains (6.8%) belong to this serotype. The study of streptococcal strains isolated from pigs will continue with molecular genetic assays for identification using q-PCR.

Key words: streptococcal infection, pigs, primary identification

VM7

THERAPEUTIC EFFECT OF ELECTROCHEMICALLY ACTIVATED AQUEOUS SOLUTIONS FOR THE TREATMENT OF INFECTIONS OF VARIOUS ORIGINS IN DOGS

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The therapeutic effect of electrochemically activated aqueous solutions (ECAAS) catholyte and anolyte with 0.3% NaCl, used alone or in combination, was investigated. The therapy was applied to dogs of different breeds with etiologically diagnosed clinical forms of dermatitis, otitis, ophthalmic and wound infections, which were treated for a long time with local or systemic therapeutic agents without a satisfactory and long-lasting effect. A number of bacterial strains identified by PhoenixTMM50 as Streptococcus anginosus, Streptococcus gordonii, Staphylococcus lugdinensis, Staphylococcus haemolyticus, Staphylococcus schleiferi, Staphylococcus simulans, Escherichia coli, Corynebacterium bovis, Enterococcus gallinarum, Enterococcus faecalis, Brevibacillus brevis, Pseudomonas aeruginosa with proven resistance to various clinically applied antibiotics have been isolated. The obtained results show that catholyte and anolyte administered alone and as the only therapeutic agent, have a well demonstrated clinical effect in the described pathological sequelae. The combination of catholyte and anolyte promotes the healing process to varying degrees depending on the peculiarities of the pathological process (chronic, acute, purulent, etc.), its localization and the phenotype of the specific etiological factor (resistance, biofilm formation, etc.). The catholyte administered in a combined regimen before anolyte resulted in a more favorable clinical effect in chronic bacterial otitis, due to the ability of catholyte to destroy bacterial biofilms. Key words: electrochemically activated aqueous solutions, anolyte, catholyte, otitis, conjunctivitis, wound infections, dogs.

VM8

DETECTION OF SOME GENES ENCODING ANTIBIOTIC RESISTANCE AMONG *STAPHYLOCOCCUS AUREUS* ISOLATES FROM COWS WITH MASTITIS IN BULGARIA

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The broad use of antibiotic drugs for treatment and control of udder infections in cows during the last years resulted in increased number of reports documenting various rates of isolation of resistant *Staphylococcus aureus* strains, including multiresistant ones, in different countries. The present research was motivated by the scarce studies at the phenotypic level on the sensitivity of bovine mastitis *Staphylococcus aureus* and the lack of data on the prevalence of genetic determinants of resistance of these isolates in Bulgaria. The purpose of the study was adaptation of PCR protocols for detection of genes encoding antibiotic resistance in *Staphylococcus aureus* isolates from cows with clinical and subclinical mastitis and estimation of agreement of genes' presence with the phenotypic resistance of isolates by means of Cohen's kappa statistic. The study was conducted with 92 *Staphylococcus aureus* isolates confirmed by *nuc* gene based PCR. Minimum inhibitory concentrations of ten antimicrobial drugs were determined on Sensititre mastitis plate format. Conventional protocols for detection of genes *blaZ*, *mecA*, *ermC*, *ermB*, *tetK* and *tetM,* either singleplex or multiplex were developed. The multiresistant MRSA DSM 29134 served as positive control in experiments. The singleplex PCR analysis demonstrated amplification products with size 517 bp, 310 bp, 190 bp and 169 bp, corresponding to *blaZ, mecA*, *ermC* and *tetK genes,* respectively. The results from the subsequent multiplex PCR assay corresponded entirely to those of the singleplex design. Out of the 29 isolates resistant to penicillin, all possessed the *blaZ* gene. *BlaZ* gene was not detected in 61 out of all 63 penicillin-sensitive isolates. The sensitivity and specificity of PCR assay for detection of resistance to penicillin were 100% and 96.8%, respectively. Among the 12 isolates resistant to erythromycin, the *erm* gene was found out in 7, it was also detected in two of susceptible isolates; therefore the sensitivity of PCR for detection of resistance to erythromycin was 58.3%, and specificity – 97.5%. With regard to resistance to methicillin and tetracycline, both the sensitivity and specificity of the reaction were 100%. The agreement between phenotypic resistance to penicillin and the presence of the *blaZ* gene was almost perfect with kappa coefficient 0.951; for erythromycin – moderate with kappa 0.625; for methicillin and tetracycline– kappa 1.000; almost perfect agreement. The developed PCR protocols may be successfully used for molecular monitoring of *Staphylococcus aureus* together with phenotypic tests.

Keywords: *Staphylococcus aureus*, cows, mastitis, resistance genes, detection

VM9

PILOT STUDY ON THE REPRESENTATIVES OF GENUS *VIBRIO* IN THE BULGARIAN BLACK SEA AREA

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The genus *Vibrio* include ubiquitous group of marine bacteria belonging to the *Gammaproteobacteria* class, the most diverse class of Gram-negative bacteria. As heterotrophic organisms, *Vibrio* spp. live freely in marine aquatic environments, from the bottom to the surface of the water column. Some *Vibrio* spp. are human and animal pathogens, and there are evidences that infections caused by vibrios are increasing worldwide. Little is known about *Vibrio* spp. diversity and abundance in Bulgarian aquatory. The main objective of this study is to gather information regarding the ecology of *Vibrio* species and their potential health risk for human and animals both. During the summer of 2021 sea water samples from different sites of Bulgarian Black Sea area and at different depth were collected. The water samples were investigated for base physicochemical parametersas temperature, salinity, pH and dissolve oxygen, as well as nutrientslike nitrogen, phosphorus and silicon. Samples were collected from farmed Mediterranean mussel (*M. galloprovincialis)* also. More than 150 strains were isolated, characterized and confirmed by MALDI-TOF MS as: *V. parahaemolyticus, V. alginolyticus, V. orientalis, V. vulnificus, V. neptunious, V. aestuarianus, V. anguillarum, V. harveyi, V. navarrensis, V. tasmaniensis, V. pomeroyi*. For the first time *V. aestuarianus* was isolated from the Bulgarian Black Sea area and from the digestive gland of *M. galloprovincialis.* The diversity of *Vibrio* representatives as well as the presence of *V.vulnificus* and *V. parahaemolyticus,* in Bulgarian Black Sea Aquatory indicates that health risk exists not only for marine animals but also for humans, especially during summer season when the sea water temperature exceeds 20 - 25 °C.

Keywords: *Vibrio spp*; Bulgarian Black Sea area; human and animal pathogens; health risk

VM10

CHANGES IN THE ACTIVITY OF ASPARTATE - ALANINE AMINOTRANSFERASE IN DOGS WITH EXPERIMENTALLY INDUCED *STAPHYLOCOCCUS AUREUS* INFECTION

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The main purpose of this study was to evaluate the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) plasma concentrations in dogs with experimentally-induced Staphylococcus aureus infection. Correlations between AST, ALT and Respiratory Rate (RR), Pulse Rate (PR) and Internal Body Temperature (IBT) were also calculated. Bacterial suspension with density of 3.1 x 109 cfu/mL was subcutaneously injected to 9 mongrel 2 years old male dogs whereas 6 other dogs served as negative controls. The concentrations were determined using commercial kits before application (0 h), 6, 24, 48, 72 h and 7, 14, 21 days after. The aminotransferase concentrations were higher in infected dogs than in the controls - AST peaked on days 7 and 14, and ALT - at the 72nd h. Strong positive correlations were recorded between ALT and AST concentrations and between RR and IBT. It was observed that the transaminases activities were slightly affected by the experimentally induced staphylococcal infection in dogs.

Keywords: *Staphylococcus aureus*, AST, ALT, clinical signs, dogs

VM11

IN VITRO ANTIBACTERIAL EFFECT OF *LEMNA MINUTA*, *CHLORELLA VULGARIS* AND *SPIRULINA* SP. EXTRACTS AGAINST FISH PATHOGEN *AEROMONAS HYDROPHILA*

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The aim of the present study was to test extracts from *Lemna minuta, Chlorella vulgaris* and *Spirulina* sp. against fish pathogen *A. hydrophila*. The water extract of these three aquatic plants were prepared. The plants were extracted in water solution at proportion 1:10. The received homogeny solutions were filtered and centrifuged at 7000 rpm for 30 minutes. Afterwards the extracts were filtered with sterile syringe filters with the size 0.2 μm. The bacterial strain of *Aeromonas hydrophila* (ATCC 7965) was used in current research. The bacterial activities of plant extract against *A. hydrophila* were tested with disk diffusion method. The aqueous extracts of all three test plants showed a good inhibitory effect against the fish pathogen. From the in vitro test conducted it was observed that the aqueous extract of C. vulgaris showed the highest zone of inhibition against *A. hydrophila* (12 mm).

Keywords: microalgae, Lemna, antibacterial effects, fish pathogen, *Aeromonas hydrophila*

Environmental Microbiology

EM1

THE CONTROL OF THE VALUABLE BIOLOGICAL MATERIALS IN AN UNSAFE WORLD

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Nowadays, more than ever, all of us live with a feeling of insecurity, both in everyday life and in professional life. As scientists and professionals in the health care field, we have to be more aware of the real threats, which unfortunately contribute to this feeling. We are talking about the biological materials (pathogens or non-pathogenic microorganisms, toxins, genetic materials, GMOs, foods, cell components etc.) and the information about these, which are potential harmful, with dual use or not (eg. for industrial, medical or research interest - with economic value or simply for historical value). All these are under the definition of the Valuable Biological Materials (VBMs). Introduced by WHO in 2006, this concept has been used when the preventive measures were set up mainly against theft, misuse, loss, unauthorized access, diversion, or intentional release of VBMs as biological weapons or for bioterrorism. Much less it was used when beneficial health, food, or environmental purposes were pursued. But, regardless of the purpose, how can we control VBMs in an efficient manner today? The approach must be based on a professional and systematic biorisk assessment of each activity and facility which implies the VBMs presence and the potential (intentional or not) release and exposure of people, animals or environment to them. We discuss here about the measures covering administrative oversight, accountability, protection, biorisk mitigation, monitoring, which means "biorisk management". A systematic review of inventory, activities, and containment measures of VBMs, along with raising awareness, periodic vetting, training, and assessment of people having access to VBMs are a challenge now, but they have to become a good common practice, based on regulatory framework, guidelines, policies and standard procedures, adapted to local situations, being able to face any changes.

EM2

TWO-STAGE ANAEROBIC DIGESTION OF ORGANIC WASTES: STATE OF THE ART AND SOME PERSPECTIVES

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Anaerobic digestion (AD) is a biotechnological process, in which microorganisms degrade the complex organic matter into simpler components under anaerobic conditions to produce biogas and fertilizer. This process has many environmental benefits, such as: green energy production; organic waste treatment; environmental protection, and greenhouse gas emissions reduction. It has long been known that the two main species (acidogenic and methanogenic) in the community of microorganisms in anaerobic digestion differ in many aspects and the optimal conditions for their growth and development are different. Therefore, in AD in a single bioreactor (single-stage process), the optimal conditions are selected taking into account the slow-growing methanogens at the expense of fast-growing acidogens, which affects the efficiency of the whole process. This has led in recent years to the development of two-stage AD (TSAD), in which processes are divided into a cascade of two separate BRs. It is known that this division of the processes into two consecutive BRs leads to significantly higher energy yields for the two-phase system (H_2 +CH_4), compared to the traditional single-stage CH_4 production process. The present paper aims to review the literature and our own studies in the field of two-stage anaerobic digestion of organic wastes. All important features of the TSAD have included: feedstocks (substrates), reactors configuration for biohythane production, process parameters affecting biohythane production, microbiology, mathematical modeling, advantages and disadvantages. Some perspectives for studies and practical implementation of the TSAD are presented as well.

Keywords: two-stage anaerobic digestion, hydrogen, methane, mathematical models

EM3

MULTIFUNCTIONAL ALGOLOGY LAB FOR MAXIMUM CO_2 CAPTURE AND SYNTHESIS OF HIGH VALUE PRODUCTS. MODERN CHALLENGES OF CLIMATE CHANGES AND VIRUS PANDEMIC

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Microalgae culture and its potential for industrial applications in CO_2 fixation from flue gases for second generation biofuels and many other valuable metabolites' production has received much attention in recent years. The lecture aims to combine all the key knowledge of the team obtained during the last 20 years in the field. The central point is creation of multifunctional laboratories in Bulgaria and Brazil for culturing microalgae on CO_2 from waste industrial gases and the engineering approach for isolation and identification of targeted microalgal strains. Special attention will be given on a new theory created by us for modeling and design of photobioreactors (PBRs) as a system and key unit operation in a technological scheme. In the light of the integral biorefinery concept, analysis of high value products obtained from algal biomass and their antimicrobial activity will be discussed in details. The results from our experience highlighted new horizons for building strategies in microalgal culturing techniques, design of nutrients media and PBRs as well as the possibilities of applications of cell products in different industries. Climate changes and their connection with virus pandemics as a new serious challenge and most importantly a danger for human society existence and life is going to be discussed, as well.

EM4

METABARCODING CHARACTERISATION OF THE BACTERIAL DIVERSITY WITHIN THE THALASSOHALINE ENVIRONMENT OF THE SALINE DI TARQUINIA, ITALY

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The "Saline di Tarquinia" (ST) are marine salterns located at ca. 80 km NW of Rome, in the North Tyrrhenian Sea area. Their microbial diversity has been scarcely studied in the past and this work represents a part of a broad investigation started in 2010 that is still in progress. In the present study, we analysed the bacterial communities found in waters samples collected along the ST salinity gradient, from the marine coastal area (sweater input to the salterns) through different ponds. The two-year survey evidenced that the number of taxa recognised in the coastal area was higher than that in the ponds. Furthermore, ST communities showed an increasing simplification of bacterial diversity accompanied by the occurrence of a few dominant taxa along the salinity gradient. Among the 38 assigned phyla, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were the most represented taxa. At the highest salinities, a preponderance of *Proteobacteria* was observed, unlike what has been usually reported for other marine salterns where the dominance of *Bacteroidetes* has been evidenced. At the genus level, the most abundant taxa were *Pontimonas*, *Marivita*, *Spiribacter*, *Bordetella*, *GpVII,* and *Lentibacter*. The α- and β-diversity analyses evidenced that the bacterial communities were highly uneven and indicated that they were structured by various factors (sampling site, sampling year, salinity, and sampling month). Among the recognised taxa, particular attention was given to *Pontimonas,* which is currently described as a genus including only one species of slightly halophilic marine bacteria. The occurrence of *Pontimonas* and its representative OTUs (OTU 1, OTU 2, and OTU 3194) was investigated along the salinity gradient as well as in a brine storage basin (BSB) located within the ST. *Pontimonas* was higher in the ponds than in the sea, whereas it had similar abundances in the sea and in the BSB. Its representative OTUs showed significant trends according to different parameters. Along the salinity gradient, OTU 1 abundance increased with decreasing water temperatures and raising rainfalls, and it showed a maximum in January; OTU 2 increased with increasing BOD_5 and it showed the highest abundances in the period August–October, and OTU 3194 increased with decreasing salinities. In addition, OTU 3194 showed a significant seasonal variation in BSB; it started increasing in spring to reach a maximum in summer. These observations indicate that *Pontimonas* can harbor broad euryhaline members and some possible extreme halophilic representatives, which can easily settle in hypersaline habitats.

Keywords: Saline di Tarquinia, bacterial communities, marine salterns, salinity gradient, metabarcoding survey

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EM5

COMPOSITION AND ANTIBACTERIAL EFFECT OF WATER FROM MINERAL SPRINGS IN THE REGION OF VELINGRAD

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As a rich and important water resource on the territory of Bulgaria water from mineral springs is widely used for drinking and therapeutic purposes. However, its composition is not completely determined and its potential for healing and cosmetic uses is not fully achieved. Therefore, the main objective of our study is to determine the composition of water from several mineral springs, test their antimicrobial properties and compare them as to find out the best ones to incorporate in medicinal and cosmetic products. To achieve this, samples from 8 mineral springs were subjected to elemental analysis for the determination of 70 elements using inductively coupled plasma-mass spectrometry and were compared to previously obtained data. Results showed that previously obtained data is not accurate and detailed enough. Concentrations of the macro elements: Mg, K, Ca and S were high in all of the samples (varying from: 0.015 to 0.155 mg/L for Mg, 3.95 to 7.25 mg/L for K, 3.83 to 15.0 mg/L for Ca and 30.9 to 56.4 mg/L for S). Elements with previously reported antibacterial, antiviral or antifungal properties (Au, Se, B, Mo, Ge, W, Sr) were also found in every sample in fairly high concentrations. The specific antimicrobial properties of the water of each mineral spring remain to be determined using agar well diffusion method and resazurin assay method. Furthermore, dry residue samples were analyzed with a Raman microscope and revealed several different crystalline structures, some of which of a sulphate nature, which presumably contribute to the healing properties of the water. The obtained information can be used for updating and expanding the data on the composition of water from mineral springs, for specifying the healing effect of mineral waters and for determining the possibilities for the use of water or their ingredients as additives to medicinal and cosmetic preparations.

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EM6

MICROBIOLOGICAL, PHYSICOCHEMICAL AND ISOTOPIC RESULTS OF GLACIER WATER FROM CHILE

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Described are the physicochemical and microbiological parameters of glacier water from glacier Mapa, Andes Mountains, Chile. The study was performed on isotopic composition with the collaboration of Oleg Mosin. Glacier Mapa is 55 km from the town Los Andes of direction Argentina. The parameters of the physicochemical composition with 18 indicators are determined according to Ordinance No. 9/2001 with document No. 12735/17.03.2016, licensed laboratory Eurotest control, Sofia, Bulgaria. The water from the given source Glacier Mapa, Chile is characterized by microbiological indicators, and the pathogenic micro-organisms in the samples from the glacier water source are determined by the methods according to Ordinance No. 9/2001, Official State Gazette, issue 30, and decree No. 178/23.07.2004 of Council of Ministers, Bulgaria about the quality of water, intended for drinking purposes. The research was conducted for *Coliforms, Escherichia coli, Enterococci, and Clostridium perfringens*. The physicochemical and microbiological parameters of glacier water Mapa, Chile correspond according to Ordinance No. 9/2001 with document No. 12735/17.03.2016, licensed laboratory Eurotest control, Sofia, Bulgaria. The natural waters consist of 99.7 mol.% of $H_2{}^{16}O$, which molecules are formed by ^{1}H and ^{16}O atoms (Mosin, Ignatov, 2012b). The remaining 0.3 mol.% is represented by isotope varieties (isotopomers) of water molecules, wherein deuterium forms 6 configurations of isotopomers – $HD^{16}O$, $HD^{17}O$, $HD^{18}O$, $D_2{}^{16}O$, $D_2{}^{17}O$, $D_2{}^{18}O$, while 3 configurations are formed by isotopomers of oxygen – $H_2{}^{16}O$, $H_2{}^{17}O$, $H_2{}^{18}O$. Studies of International standard SMOW of isotopic shift for D were performed for D/H from glacier water Mapa, Chile. The results obtained from glacier water Mapa Chile show a lower content of hydrogen and oxygen isotopes. Absence of pathogenic microorganisms. Physico-chemical indicators are characteristic of water during the melting of ice in glacier water (Ca^{2+} 6.0; Mg^{2+} 1.0; Na^{+} 5.0; K^{+} 0.38; HCO_3^{-} 73.8 /mg. L^{-1}). The water from Glacier Mapa is useful for health in accordance with the obtained physicochemical, microbiological, and isotopic parameters.

Keywords: Glacier water, Glacier Mapa, Andes Mountains, Chile, physicochemical and microbiological indicators, isotopic composition

EM7

INFLUENCE OF URBANIZATION ALONG THE YANTRA RIVER ON THE PREVALENCE OF ANTIBIOTIC RESISTANT BACTERIA

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Antimicrobial resistance (AMR) is one of the major threats to human health and is becoming an environmental challenge for water resources too. Urbanization along the rivers (settlements and human activities) presupposes the emergence and dissemination of water pollutants, including bacteria resistant to various antimicrobials. *The aim* of the study was to assess the prevalence of antibiotic resistant bacteria in individual sections of the Yantra River exposed to different level of urbanization and impact of human activities. Culture-dependent methods were used for water analyses. Surface water samples were collected from ten sampling points along the Yantra River and were analyzed for enumeration of HPC bacteria and the fraction of the bacteria resistant to antibiotics from different classes. The *E. coli* number was also determined, and 68 strains were isolated and subjected to susceptibility testing towards 14 antibiotics. The study carried out initiated the investigations of the level and profile of AMR of the the Yantra River microbiome. It was found that the proportion of bacteria resistant to different classes of antibiotics varied in the river sections, reflecting the anthropogenic impact of urban areas. In the river section before the town of Veliko Tarnovo, the lowest values of AMR to the different antibiotics were established, and the river crossing through the town did not have a significant negative effect. The confluence of the Belitsa River contributed to an increased AMR towards some antibiotics. In the river waters, the population fraction of the heterotrophic bacteria resistant to tetracycline was the lowest (2.4%), and the highest - of those resistant to ciprofloxacin (14%) and chloramphenicol (11%). The phenotypic profile of the isolated *E. coli* strains showed that 65% of them were resistant to at least one antibiotic, and 12% were multidrug resistant toward three or more antibiotic classes. The largest number of isolates were resistant to ampicillin (18%), and the smallest - to gentamicin (3%).

Keywords: antibiotics, antimicrobial resistance; antibiotic resistant bacteria; surface water; *E. coli*

Acknowledgements: The research was carried out within the scope of the Project BG05M2OP001-1.002-0019 „Clean technologies for sustainable environment – water, waste, energy for circular economy“ (Clean&Circle) for development of a Centre of Competence, financed by the Operational programme “Science and Education for Smart Growth” 2014-2020, co-funded by the European union through the European structural and investment funds.

EM8

ANTIMICROBIAL RESISTANCE OF DRINKING WATER AND DRINKING WATER-ASSOCIATED BIOFILMS

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The increasing antimicrobial resistance (AMR) among pathogens is a serious health risk that in recent years, has grown into an ecological challenge for water environment, due to the emerging of undigested quantities of antibiotics and bacteria that have acquired AMR. The so called Urban Water Cycle, which includes drinking water supply, sewerage and wastewater treatment, predefines the social importance of the interrelation between AMR and water. Sharing different aquatic habitats and moving between wastewater, natural and drinking waters determine the role of antibiotic resistant bacteria as carriers or potential vectors of AMR towards human microbiome. The main objectives of the study were to evaluate the fractions of antibiotic resistant bacteria in drinking water and biofilms from a selected drinking water supply system (DWSS) and determine the antibiotic susceptibility profiles of isolated bacterial strains. Culture-dependent methods were used for microbiological studies. The relative fractions of the bacteria resistant to individual antibiotics were assessed. Bacterial strains were isolated and tested for susceptibility to eight antibiotics by the disc diffusion method. Population studies of the drinking water microbiome to nine selected antimicrobial substances were carried out. The populations' dynamics of the bacteria resistant to individual antibiotic at two sampling points at the DWSS was studied. The total AMR of 74 bacterial strains was determined. The largest share of isolates was resistant to beta-lactams (ampicillin - 32% and cefotaxime - 32%), and the smallest one - to tetracycline (1%) and ciprofloxacin (7%). Significant differences were found in the phenotypic AMR profile of bacterial strains isolated from the both DWSS sampling points: 51% of the isolates from point № 1 were resistant to at least one antibiotic, while at point № 2 - 29%; 5% of the isolates from point № 1, but 18% of those isolated from point № 2, were multidrug resistant.

Keywords: antimicrobial resistance; antibiotic resistant bacteria; biofilm; drinking water; drinking water supply system

Acknowledgements: This research was funded by the National Science Fund, Republic of Bulgaria (grant number: КП-06-Н31/20 from 17 December 2019).

EM9

SCIENTIFIC PROJECT "CAVES AS A RESERVOIR FOR NOVEL AND REOCCURRING ZOONOSES - ECOLOGICAL MONITORING AND METAGENOMIC ANALYSIS IN REAL TIME"

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Bats are the second largest order of mammals and a reservoir of viral, bacterial and fungal pathogens, some of which are particularly dangerous. Caves offer environment with extreme conditions – lack of light, less nutrients, high humidity, low temperature and others, which leads to high level of adaptation of the organisms. This fact is prerequisite for higher mutation level and as a result microorganisms inhabiting caves have higher probability to overcome species barrier. Bats physiology and their environmental preferences modulate a microbial flora with unpredictable potential. There are no detailed studies of Bulggarian caves which characterized the metagenomic diversity of cave dwellings (as bats and blood-sucking insects) and their environment (water and soil). Aim of our project is to develop and test an innovative approach for monitoring and predicting epidemic outbreaks of new and emerging pathogens in the environment in real time, by applying genome sequencing and bioinformatics analysis. Samples from three caves - Orlova Chuka, Devetashka and Parnitsite were collected since the spring of 2022. DNA was isolated measured and prepared for NGS analysis. Targeted 16S and ITS NGS analysis was applied using Illumina next generation sequencers. Output of the sequencers was obtained in FASTQ files. The sequenced samples were analyzed with metagenomic sequence software tools, i.e. Geneious, OTU annotations and phylogenetic analysis were performed by specific bioinformatic tools, i.e. Unicycler, Prokka and others. Analysis of caves water and soil, bats' blood and tissue samples is in progress. Fighting the spread of new and emerging infections is a global problem and a significant societal challenge. Our results will expand the range of methods for assessing the risk of potential future epidemics.

Keywords: caves, microbiome, bioinformatics

Acknowledgements: This work is funded by the Bulgarian National Scientific Fund, grant КП-06-Н51/9 of 16.11.2021.

EM10

APPLICATION OF BACTERIOPHAGE-BASED BIOPESTICIDE IN RHIZOSPHERE SOIL AND INFLUENCE ON BENEFICIAL SOIL MICROORGANISMS

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The increasing number of multidrug resistant bacteria challenge the scientist for studying and developing of alternative bactericidal preparations. Over the last several decades, the potential of bacteriophages, the natural enemies of bacteria, in combating bacterial diseases, is studied very extensively. Thus, the aim of our work is to study the potential of three bacteriophages (BsXeu269p/3, BsXeu105t/1 and BsXeu105t/4), isolated from Bulgarian soil, in combating bacterial spot disease on tomato and pepper plants. The phytopathogenic bacteria, causing the disease, are members of the genus *Xanthomonas* and the main target for these bacteriophages is the species *Xanthomonas euvesicatoria*. The bacteriophages, were isolated in 2018 from rhizosphere soil of infected tomato plan in Bourgas, Bulgaria. Our main goal was to: 1/ study the influence of different pH (2-13) on phage viability of the three isolates and 2/ to measure the time limit of phage survival in soil sample without the presence of the specific host and the influence on beneficial rhizosphere microorganisms (heterotrophs, nitrogen fixing bacteria, ammonifying, nitrifying and denitrifying bacteria) of one selected phage isolate (BsXeu269p/3). Bacteriophages were incubated in buffers with corrected pH for 48h and the differences in phage titers (initial and after 48h) were measured via double agar overlay plaque assay (DAOPA). The viability of the phage BsXeu269p/3 in soil sample was determined after measuring of number of viable phage particles after 62 days of incubation. Its influence on soil microbiota was determined after direct infecting of the different soil bacteria and observing for presence/absence of lysis. The three tested phage isolates remain 100% their viability and capability to infect the target bacterium (*X. euvesicatoria*) under acidic conditions (2, 5 and 5,92). The incubation in neutral and alkaline buffers (7,5; 9 and 11) led to slide decrease (up to 1 lg) only in the titers of phage isolate BsXeu269p/3. The only pH value we found out to have immediate phagocidal effect was 13. The phage survival in soil sample was determined at 55th and 62th days post soil inoculation with phage suspensions. Viable phage particles were found only at 55th day of the experiment but their amount was extremely low (titer decreased 8 lg). We found that phage isolate BsXeu269p/3 is not capable to infect cells of heterotrophs and nitrogen fixing bacteria as no indication for lysis was observed after cultivation. The addition of viable phages in the cultivation media of ammonifying and denitrifying did not affect them and their number remained 100% compared to the initial number measured. The obtained results indicated that the Bulgarian bacteriophages have good potential as biocontrol agents as they meet the crucial criteria as viability in soils with different pH, long-term viability without their specific host in soil and last but not least – do not affect the accompanying microflora of the plants.

Keywords: bacterial spot diseases, phage biocontrol, *Xanthomonas euvesicatoria*

EM11

DIURNAL VARIATIONS IN THE CONCENTRATION OF MICROBIAL BIOAEROSOLES IN OUTDOOR AIR OF HIGHLY URBANIZED SOFIA CITY CENTER

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According to the World Health Organization, air pollution is the most significant environmental risk factor for the population of the European Union. At the same time, the main scientific interest for decades has remained focused mainly on physical and chemical air pollutants, which severely limits our knowledge of microbial contamination in the outdoor air. The exploration of the complex nature of air pollution has become apparent in recent years, and efforts are focused on research related to establishing the qualitative and quantitative composition of microbial bioaerosols, as well as the complex nature of its dynamics. The main goal of the present study is the quantification of diurnal dynamics of outdoor air bioaerosol contamination over the highly urbanized central part of Sofia city. The sampling location is the Faculty of Biology, Sofia University. The exact coordinates are as follows: Sofia, Dragan Tzankov 8 Blvd., GPS coordinates: 42 ° 41'01.9 "N, 23 ° 19'58.3" E. *In situ* sampling was achieved by Six-Stage Viable Cascade Impactor (FSC-A6 (Honri Air Clean Technology Co., Ltd), for size-selectively enumeration of culturable heterotrophic bacteria and fungi in the range from >7μm to 0.65μm. Four days from all the seasons with typical seasonal abiotic parameters of the environment were selected for sampling. The reported daily dynamics in the bacterial and fungal bioaerosol concentrations demonstrate a different profile depending on the season. The concentrations of the fungal and bacterial bioaerosols in the outdoor air of the location change hourly, in direct connection with the abiotic parameters of the environment and anthropogenic pressure. At the first interval (7:00) the bacterial and fungal bioaerosol concentrations were as follows: 506/819 CFU/m^3(summer); 422/165 CFU/m^3(autumn); 18/17 CFU/m^3(winter); 219/646 CFU/m^3(spring). During the noon (13:00 PM) the simultaneous decrease in the concentrations of bacterial and fungal bioaerosols was observed in the summer and spring seasons. At the same time in autumn and winter, there is an increase in both types of bioaerosols (with maximum daily values reached during the winter season). During the summer season daily minima for both microbial bioaerosols were registered at 16:00. During the autumn season, the levels of bacterial bioaerosols decrease, while the maximum daily concentration of fungi is reported. The dynamics are similar during the same interval in the winter season, while in the spring there is an increase in both types of bioaerosols in the afternoon. During the summer season at 19:00 o'clock, there was an increase in the reported values for microbial bioaerosols in outdoor air, with a daily maximum for fungi. For the same interval during the autumn season, an increase was registered for bacterial bioaerosols, while there was a decrease in fungal concentration. It could be concluded the daily variations in the concentrations of the microbial bioaerosols in the air is different for all the seasons and follows the changes in anthropogenic activity. Significantly high levels were found in the morning, followed by a reduction in values at noon and a secondary increase in bioaerosol concentrations in the afternoon.

Keywords: microbial bioaerosols; diurnal dynamics

EM12

EXTRACELLULAR SIALIDASE ENZYME PRODUCTION BY FILAMENTOUS FUNGI FROM LIVINGSTON ISLAND, ANTARCTIC

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The enzymes of the sialidase family are widely distributed in nature, including viruses, protozoa, bacteria, fungi, vertebrates, and higher animals. These enzymes hydrolyze terminal *N*- or *O*-acylneuraminic acids, which are α2,3-, α2,6-, or α2,8-linked to glycoproteins, glycolipids, polysaccharides, mucopolysaccharides, and oligosaccharides. Bacterial sialidases have been considered virulence factors in many pathogenic organisms, including *Vibrio cholerae, Streptococcus pneumoniae, Clostridium perfringens, Pseudomonas aeruginosa, Klebsiella aerogenes* etc. In viruses with virulent qualities, this enzyme could be found in paramyxoviruses and orthomyxoviruses. There are only isolated reports for such enzymes in fungi. Moreover, the availability of sialidase has not been studied in fungi adapted to extremely cold environments. The aim of this study was to determine the distribution of the sialidase enzyme among fungal strains from Livingston Island, Antarctica. Sialidase activity was assayed in the cultural filtrate of 34 fungal strains belonging to the Mycological collection of the Stephan Angeloff Institute of Microbiology. They were isolated from the vicinity of the permanent Bulgarian Antarctic base "St. Kliment Ohridski" (62° 38 '29 "S, 60° 21' 53'' W) on Livingston Island (South Shetland Islands, Maritime Antarctica). The strains were identified by molecular biological methods. For screening of sialidase activity, cultivation medium AN3was used. The fungal strains were cultivated in 500 mL Erlenmeyer flasks containing 74 mL medium and 6 mL spore suspension at a concentration of $2x10^8$ spores/mL at 25°C for 72 h on a rotary shaker (220 r.p.m.). The effect of different parameters on growth and enzyme activity was evaluated. The result showed that enzyme activity was found in culture filtrates from 31 strains, while only three did not exhibit sialidase synthesis. Sialidase production was established in strains belonging to 8 genera. The most active strains were found in the genera *Penicillium*, *Aspergillus, Cladosporium, Alternaria, Talaromyces,* and *Mucor*. The highest enzyme activity (8.813 U/ml) was demonstrated by *P. griseofulvum* P29, followed by *A. fumigatus* 1-9, *P. fimorum* III 7-1, and *Penicillium* sp. P22 (6.875, 6.688, 6.625 U/ml, respectively). The ability of fungi to produce sialidase was confirmed by the fluorescent assay against 4MU-Neu5Ac. The strain *P. griseofulvum* P29 was chosen as a better producer of sialidase. Maximum enzyme activity was obtained in a 3L bioreactor after 48 h cultivation on a medium supplemented with 0.5% milk whey at a temperature of 20°C.

Keywords: sialidase/neuraminidase, fungi, Livingston Island, production

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EM13

PRELIMINARY STUDY OF GENETIC DIVERSITY AMONG ROSA GENOTYPES BY ISSR MARKERS

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The genetic relationships among different Rosa species and *Rosa damascena* genotypes from various cultivation areas in Bulgaria and Syria were analyzed using Inter Simple Sequence Repeats primers. The nine ISSR primers produced a total of 128 bands across all studied genotypes, of which 116 were polymorphic and twelve were monomorphic. Percentage polymorphism ranged from 55% with primer (GA)8T to 100% with primers (AC)8G and (AG)8T. The number of amplified bands varied from nine to twenty-two. Polymorphic information content, effective multiplex ratio, resolving power, and marker index were calculated for all primers. These results were supported by Neighbor joining and principal coordinate analysis (PCoA). Nei Genetic Distance and Genetic Identity showed relation among studied roses genotype. UPGMA method divided Rosa species and *Rosa damascena* genotypes into two main clusters. The genetics research of roses has economic importance and is necessary for breeding programs and for the utilization of genetic resources. Also, it provides valuable data for their evolution and inter and intra species relationships.

Keywords: *Rosa damascene*, Rosa species, ISSR markers, genetic relationship of roses, genetic resources, and breeding of roses

EM 14

FIRST INSADE VIEW ON MICROBIAL COMMUNITY OF A GRAPHITE MINE

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In the frame of a project related to degradation of Graphene-based materials (GBMs), including graphene (G) and graphene oxide (GO), it is of fundamental interest to question about the potential adverse effects of these materials on organisms as well as their persistence in the environment. Despite the abundant bibliography focused on GBMs, there is still a limited number of studies reporting *in vivo* biological degradation of this type of two-dimensional nanomaterials. The aim of the project is to explore the ability of environmental bacteria to degrade and to interact with G and GO. Two approaches were applied: 1) straightforward approach- NGS sequencing of metagenomic libraries and 2) culture dependent approach. First, samples from graphitic deposit sites were collected and the diversity of indigenous bacterial communities were deciphered via metagenomic analyses of 16S rDNA extracted directly from the sample. Further, was applied NGS technology, where the V3-V4 region of the 16S rDNA gene was used to create metagenomic libraries with at least 400bp length. Bioinformatics analyses for identification of presented bacterial and archaea species were performed at NCBI and RDB.

The culture-dependent approach includes enrichment cultures for strict anaerobes, performed at different abiotic conditions (T, pH), diverse growth media, etc. Overall on 2 mineral nutrient media [1] 243 enrichment cultures were performed at 3 different temperatures with 3 different trace element solutions [2,3], and 3 different electron acceptors. The electron donor is GO. In addition, several abiotic parameters of the mine samples important for bacterial growth-total C, organic C, total Nitrogen, TKN, etc., were measured. Here are presented the results from one graphitic mine located in South Africa- Karoi. This is the first study on microbial communities from graphitic mines, so far.

Keywords: graphitic mine metagenomics, graphene oxide bacterial interactions

Acknowledgements: This work is in the frame of EU program FLAG-ERA, supported by the BNF, Project DeGraph, КП–06–Д002/6.

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Virology

V1

CORRELATION BETWEEN HBSAG QUANTITATIVE ASSAY RESULTS AND HBV DNA LEVELS IN CHRONIC HEPATITIS B PATIENTS – A SINGLE-CENTER EXPERIENCE

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Active viral infection can be detected by quantifying HBV DNA, but such assays are molecular-based and often expensive for the health systems worldwide. Viral load has been used to diagnose and monitor patients who are being treated for chronic hepatitis B (CHB), which is still a major health problem, considering the lower-intermediate seroprevalence rate of HBsAg in Bulgaria. A cheaper laboratory test can be used as a substitutional marker for the molecular detection of HBV DNA. Quantitation of hepatitis B surface antigen (HBsAg) by automated chemiluminescent immunoassay (CLIA) has been proposed to be a surrogate marker that might simplify our management. We determined whether quantitative HBsAg levels correlate with HBV DNA levels in CHB patients.

In this cross-sectional study, all 228 CHB patients who were referred by a gastroenterologist to undergo quantitative HBV DNA assay in a qualified laboratory in University Hospital "St. Marina" Varna, Bulgaria from January till May of 2022 were enrolled. Serum samples were obtained. HBV DNA was measured by real-time polymerase chain reaction (Sacace Biotechnologies, Italy), and serum HBsAg was quantified by chemiluminescence assay (Dia Sorin, Italy). Additionaly HBeAg was semi-quantitatively measured by ELISA (Dia Pro, Italy) for 66 of the patients. Out of 228 patients, 139 were male (61%) and 89 were female (39%); the mean age was 55.9 ± 13 years. 190 patients were with at least one positive result (HBV-DNA or/and HBsAg) and 38 patients with both HBsAg and HBV-DNA negative (they were excluded from further analysis). By Spearman test, there was a week-moderate correlation between HBsAg and HBV-DNA ($r = 0.312$ and $p<0.01$). Approximately a quarter of the patients (n=66) were tested additionally for HBeAg and 90,9% of them were HBeAg-negative. By Mann-Whitney U test, HbsAg titer differed significantly between HBeAg-positive and -negative patients ($p = 0.006$), as did HBV DNA levels ($p = 0.01$). By chemiluminescence assay, HBsAg levels had week-moderate correlation with HBV DNA levels in CHB with predominant HBeAg negativity. HBeAg-negative patients have lower levels of HBsAg and lower levels of HBV DNA.

Keywords: HbsAg quantitative, HBV DNA, CLIA, PCR, CHB

V2

ANALYSIS OF THE TRANSMITTED DRUG RESISTANCE AND MOLECULAR EPIDEMIOLOGY OF HIV-1 IN BULGARIA, CHALLENGES AND PROSPECTS

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Modern antiretroviral therapy has turned HIV infection from a fatal disease into a chronic one. However, evolving resistant mutations can compromise antiretroviral treatment. Rapidly growing local outbreaks in vulnerable groups can accelerate the spread of resistant mutations and compromise the first line therapy regimen. The aim of the study was to analyze transmitted drug resistant mutations (TDRM) and to identify phylogenetic clusters with these mutations among people living with HIV/AIDS in Bulgaria. The National Reference Confirmatory Laboratory for HIV (NRCL of HIV) provides virological monitoring of all individuals with HIV/AIDS in Bulgaria. A wide range of methods were used to analyze resistance mutations to antiretroviral therapy using state-of-the-art methods in molecular biology. By sequencing different fragments of HIV polymerase gene, resistant mutations to different classes of antiretroviral drugs were analyzed. A variety of bioinformatics programs have been used to conduct molecular epidemiological analysis and to reconstruct phylogenetic trees.

For 35 years from the beginning of the HIV / AIDS epidemic to the end of 2021, 3697 people were diagnosed with HIV in Bulgaria. Of these, 2991 (80.9%) were men, 1755 (47.5%) were infected through heterosexual transmission and the rest belonged to other transmission groups. Successful antiretroviral resistance tests have been performed on 2473 patient samples. Resistant mutations to various classes of antiretroviral drugs have been identified, including nucleotide/nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, CCR5 antagonists and integrase inhibitors. Significant numbers of resistant mutations have been identified in patients on therapy as well as in naive untreated individuals. Phylogenetic clusters with resistant mutations have been identified in newly diagnosed individuals with treatment failure.

Modern antiretroviral therapy is largely able to control the disease. However, resistant mutations can cause treatment failure, therefore continuous monitoring of viral load and resistance mutations is required. Phylogenetic clusters with resistant mutations demonstrate the potential for accelerated dissemination of resistance, which can result in first-line therapy failure.

Keywords: HIV/AIDS; resistance mutations; subtypes; phylogenetic clusters

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V3

GENE SILENCING OF SOME VIRAL GENES OF HUMAN COXSACKIEVIRUS B

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Enteroviruses are the most common human viruses, causing hundreds of thousands of hospitalizations annually in the developed world. Some of the diseases lead to severe consequences of the central nervous system, the heart, diabetes, etc. Chemotherapeutic agents with specific anti-enteroviral activity for clinical use are lacking. We used the NCBI genetic database to analyze the presence of conserved regions in the viral genomes of Coxsackie B viruses compared to the CVB1 and CVB3 virus collection of the Institute of Microbiology at the Bulgarian Academy of Sciences. For this we used Clustal Omega program. As targets in the analysis, we selected relatively conserved regions of structural proteins (VP1, VP3), key for virus replication, non-structural proteins (2A, 3C, 3D), which are responsible for blocking the replication cycle of CVB, as well as non-coding regions such as the 5'UTR region, which is involved in translation initiation, turning off the translation of cellular mRNAs. Conserved regions of the CVB genome were identified as target regions for gene silencing, dsRNAs and pools of overlapping siRNAs were obtained. Their cytotoxicity against HEp-2 cells was tested, as well as their antiviral effect on CVB. Full inhibition of viruses was achieved at non-toxic concentrations of siRNAs. The dependence of the antiviral effect of dsRNAs and siRNAs on the multiplicity of infection of CVB1 and CVB3, as well as the dependence on the transfecting agent, was tested. The effects of dual combinations of siRNAs were investigated. The obtained results are completely original and reveal new perspectives in the therapy of enterovirus infections.

Keywords: Coxsackievirus B, PTGS, 5'UTR , siRNAs

V4

SPATIO-TEMPORAL DYNAMICS OF CO-CIRCULATING RABIES VIRUS VARIANTS ISOLATED IN DOG POPULATION IN CAMEROON

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Rabies is a widespread zoonosis in almost every country in the world and causes an estimated 60,000 human deaths each year (1, 2). As the dog is the main vector of rabies in humans, mass vaccination campaigns of dogs with a vaccination coverage of 70% of the canine population have allowed the elimination of human rabies in certain epidemiological contexts. The etiological agent of rabies, the *Rabies Lyssavirus* or Rabies Virus (RABV) belongs to the genus *Lyssavirus and* family *Rhabdoviridae* (3). Previous studies on molecular characterisation of RABV in dog population of Cameroon have detected the sub-clades Africa 1a and 1b of the Cosmopolitan clade in the sourthern regions of Cameroon with high prevalence in the Centre region ; while the Africa 2 clade was detected with very low prevalence in the Far North, North, West, and North West (4-9). Phylodynamic studies coupled with epidemiological data have been shown to provide a better understanding of the factors that promote the spread and maintenance of rabies virus (10). However, no study has been conducted to investigate the origin of the RABV isolates detected in dog populations in Cameroon and the dynamics of the spread of the canine rabies virus in the regions of Cameroon. we determined the evolutionary history of RABV from the N, P, M and G genes of the genome. The time-scale Markov chain Monte Carlo (MCMC) phylogenetic tree was estimated with BEAST 1.10.4 softwares, using the The General Time Reversible model with proportion of invariable sites plus gamma-distributed rate heterogeneity (GTR+G+I) as the nucleotide substitution model. The latitude and longitude of the detected isolates was used to perform phylogeographic analyses, in combination with the phylogeny of the different sequences, to estimate the spread of RABV in Cameroon. The rate of evolution of isolates detected in Cameroon was 5×10-4 substitutions per site, per year. The TMRCA of sequences belonging to clade Africa 1a was estimated between 1981-1991 (95% HPD; average of 1986), while those of clade Africa 2 were estimated between 1976 and 1986 (95% HPD; average of 1981). Overall, phylogeographic analyses showed that the city of Yaoundé was the main focus of RABV infection and that RABV spread between cities near and far in Cameroon through the movement of infected dogs, facilitated by human movement.

Keywords: Rabies virus, dog, phylogenetic, phylogeographic

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V5

BRONCHIAL EPITHELIAL CELLS AT THE AIR-LIQUID-INTERFACE FOR INVESTIGATION OF HOST-INFLUENZA VIRUS INTERACTIONS

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Influenza, like other respiratory viral infections, often leads to severe complications due to dysregulated cellular processes and abnormal immune responses. *In vivo* investigations of the molecular mechanisms underlying the anti-viral response can be laborious and time-consuming. Ciliated airway epithelium generated from primary human cells and developed as an *in vitro* culture system provides an optimized model for studying the pathogenesis of chronic and acute pulmonary disease with various etiologies. Host-virus interactions that occur in the context of underlying chronic inflammation can be elucidated *in vitro* using normal primary human bronchial epithelial (NHBE) cells differentiated at the air-liquid-interface (ALI).

Here, we present results from experiments using NHBE cells cultivated using the ALI approach. Primary NHBE cells were expanded in supplemented PneumaCult-EX Plus medium. Early passage cells (p3) were transferred onto transwell inserts in 12-well plates and kept submerged until they reached confluence. The cells were then airlifted and cultured in PneumaCult-ALI complete differentiation medium for four weeks. Differentiation-associated changes were monitored histologically over time. On week 4, half of the transwells were treated with 20 ng/mL recombinant human Interleukin 1β (IL-1β) for 24 hours to induce an inflammatory state, while the other half were left untreated. The cells were then infected with Influenza A/H3N2 virus at 1 m.o.i. Four hours post-infection the cells in each transwell were used for extraction of total RNA. Downstream analysis of host mRNA was used to reveal transcript differences among influenza-infected, pro-inflammatory cytokine challenged but non-infected, and double-hit IL-1β-treated and infected epithelium compared to healthy NHBE-ALI cultures.

Differentiated human bronchial pseudostratified epithelium in ALI culture could be a cost-effective tool for studying the pathogenesis of chronic inflammatory illnesses of the lung, such as COPD, asthma, and cystic fibrosis, but also of acute pulmonary infections, such as the flu and COVID.

Keywords: NHBE cells, ALI differentiation, inflammation, influenza, host-virus interactions.

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V6

REMOVAL OF VIRUSES FROM THE AIR BY ADSORPTION WITH ACTIVATED CARBON

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Coronavirus disease 2019 (COVID-19) is one of the worst pandemics that hit the humanity recently. The manifestations are quite varied, ranging from severe lung infections to asymptomatic variants. There is an urgent need to develop new methods to decay this pandemic. The use of protective masks is one of the main preventions to deal with the Covid pandemic. Application of renewable masks is more effective than using disposable masks.

Activated carbon adsorption is widely used to purify water and air from various organic and inorganic pollutants, bacteria, viruses. In the present study, waste biomass precursors was processed into a solid product (carbon adsorbent). Different samples of biomass samples were tested, but the best characteristics were obtained for activated carbon from apricot stones. The adsorbent was synthesized by a one-step method developed in our Laboratory "Solid Fuel Chemistry" (IOCCP-BAS). The sample is subjected to hydropyrolysis at a temperature of 800^0 C for 1 h. The carbon adsorbent has been successfully tested for uptake of viruse similar to SARS-CoV-2. After 50 h all the viruses are absorbed by the filter containing activated carbon. After virus removal, activated carbon can be easily regenerated with steam and reused. The carbon adsorbent thus obtained was successfully in the production of internationally certified protective masks.

Keywords: Activated carbon, SARS-CoV-2, adsorption.

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V7

REAL TIME QUANTITATIVE PCR AS A RELIABLE METHOD FOR DETECTING CMV REACTIVATION IN PATIENTS WITH LYMPHOMAS

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CMV becomes an important pathogen in individuals whose immune system is compromised like in patients with various types of lymphoma. The aim was to determine CMV seroprevalence of 49 patients with lymphomas in Varna region, Bulgaria by ELISA and to estimate the deal of CMV reactivation in quantitative RealTime-PCR (QRT-PCR) during the treatment of the main disease. Commercial ELISA kits (Euroimmun, Germany) and Taq-man Real Time Quantitative PCR for CMV-DNA detection (Sacace, Biotechnologies, Italy) were used. Of the investigated 49 patient diagnosed with lymphoma at "St. Marina" University Hospital Varna, Bulgaria we found 90% (95% CI: 78.9% - 97%) of them anti-CMV IgG positive in ELISA. We found 10% (95% CI: 1.6% - 18.3%) of them positive in QRT-PCR (range 2 332 - 5 688 IU/ml). The highest viral loads were detected in three patients with non-Hodgkin's lymphoma, histologic variant "diffuse B-cell giant cell lymphoma".

In conclusion, QRT-PCR is reliable method for the early identification of CMV reactivation in patients with various types of lymphomas and would assist with management during the treatment of the main disease.

Keywords: Cytomegalovirus; CMV PCR; lymphomas; CMV reactivation

V8

COMBINED ANTIVIRAL TREATMENT AGAINST COXSACKIEVIRUS B3 AND POLIOVIRUS

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The human enteroviruses (EV) are ubiquitous viruses that are transmitted from person to person via direct contact with virus shed from the gastrointestinal or upper respiratory tract. Poliovirus, the prototypical enterovirus, can cause a subclinical or mild illness, aseptic meningitis, or paralytic poliomyelitis. Treatment is primarily symptomatic because there are no EV-specific drugs available for clinical use. One of the reasons is fast development of drug-resistant mutants. Combination therapy can overcome toxicity and restrict the emergence of resistance to the drug partners.

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 was 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 agains CV-B3 demonstrated 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 effect on uninfected HEp-2 cells.

Keywords: Coxsackievirus B3, Poliovirus type 1, double combinations

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V9

TREATMENT VIA CONSECUTIVE ALTERNATING ADMINISTRATION OF ANTIVIRALS AGAINST 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 consist 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 lengthening of the 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.

Keywords: Coxsackievirus B3, triple combination, mice

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V10

EFFECT OF CASTALAGIN AGAINST HSV-1 INFECTION IN MICE

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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, special interests 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 test *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 include groups receive different daily doses, 20 mg/kg, 10 mg/kg, 7.5 mg/kg or 5 mg/kg of compound. The 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. The reference course of acyclovir demonstrated a marked activity at daily dose of 20 mg/kg.

Keywords: ellagitannins, castalagin, HSV-1

V11

SARS-CoV2 SPIKE, ENVELOPED AND MEMBRANE PROTEINS EXPRESSED IN NICOTIANA BENTHAMINA DETECT SPECIFIC ANTIBODIES IN COVID-19 PATIENTS

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The ongoing Covid-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a serious health crisis with an ever-increasing number of cases. Developing alternative methods to measure seroprevalence is a priority. We developed an in-house ELISA using the full-length spike (S) protein or EMS virus-like particles (VLPs) (envelope, membrane, and spike protein) from SARS-CoV-2 expressed in *Nicotiana benthamiana*. We determined antibody responses in sera from patients (n = 35) who had tested positive by PCR for SARS-CoV-2. Serum samples were taken a median of 8 weeks after the diagnosis, and the majority of participants had moderate or severe COVID-19 disease. As a control, we analysed the reactivity of pre-pandemic plasma (n =24). We demonstrate that specific anti-SARS CoV2 immunoglobulin G are detectable using recombinant plant-derived S protein or EMS in COVID-19 patients. Reactivity to S and EMS was detected in 24 (100%) and 22 (91%) of participants, respectively. For the pre-pandemic plasma, 1/24 (4.1%) of samples were positive, indicating a high specificity for SARS-CoV-2 in our ELISA. We demonstrate that recombinant SARS-CoV-2 proteins produced in plants enable robust detection of SARS-CoV-2 humoral responses and this assay can be used for seroepidemiological studies.

Acknowledgments: The work was supported by the European Union's Horizon 2020 research and innovation programme, project PlantaSYST (SGA-CSA No. 739582 under FPA No. 664620) and by the European Regional Development Fund through the Bulgarian 'Science and Education for Smart Growth' Operational Programme (project BG05M2OP001-1.003-001-C01), Research Fund at the University of Plovdiv, competition 'Young Scientists and Doctoral Students' MU21-BF-022.

V12

DATA ON THE PREVALENCE OF HEPATITIS E VIRUS AMONG DOMESTIC PIGS AND WILD BOAR IN BULGARIA

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In the last decade in Europe, including Bulgaria, reports of Hepatitis E virus (HEV) infection in humans have increased, proving that the prevalent viral strains (genotype 3 and less often 4) are of local origin, and not imported from endemic to the virus countries. At the same time, the spread of Hepatitis E has also been confirmed in a number of animal species, domestic pigs being considered the main reservoir. It is assumed that human infection occurs after consumption of raw or poorly thermally processed meat products or during direct contact with the infected animal. From 2020, the spread of HEV in Bulgaria is being investigated more than ever before. A recent study shows slightly over ¼ of tested blood donors have proven positive for anti-HEV IgG, as the number was higher for hunters. Significant seroprevalence of HEV is detected as well in pigs, wild boar and East Balkan Swine. The first comprehensive study of seropositivity of domestic pigs for the presence of anti-HEV IgG antibodies was performed by our group and confirmed 73.65% positivity of the slaughter-aged pigs. We also reported the first presence of HEV in wild boar with 12.5% seropositivity. The next large-scale study of the prevalence of HEV in wild boar (done by Tsachev et al.) found the presence of specific antibodies in 40.8% of the examined samples. Moreover, molecular studies confirm very high similarity of strains circulating in animals and humans across the country, which assumes zoonotic transmission.

Keywords: Hepatitis E virus, HEV, pigs, wild boar, zoonosis

Acknowledgements: This research was funded by Research Fund at the University of Plovdiv, competition "Young Scientists and Doctoral Students" MU21-BF-022.

V13

ELEVATION OF LIVER ENZYMES DURING COVID-19 PANDEMIC – BULGARIAN STUDY ON 3038 PATIENTS

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By June 2022 there were over 532 million confirmed cases of COVID-19 disease and about 6 million confirmed deaths according to WHO. COVID-19 is a systemic disease with multiple organ damage, liver injury being the most common after lungs. It is still unclear what is pathophysiology of the liver damage due to COVID. When the liver is damaged, liver enzymes may leak into the bloodstream. The aim of our study was to invstigate and compare the values of liver enzymes of patients in pre-Covid and during the Covid period. Analyzes were performed at Nadejda-1 Clinical Laboratory in Sofia for a period of 3 months during autumn for 3 consequent years - 2019 (Pre-Covid) and 2020 and 2021 (Covid). ASAT, ALAT and GGT of total 3038 aged 1-80 years were examined. Analyses were carried out at automatic biochemical analysers Cobas Mira CC и Selectra Pro S, calibrated daily according to the manufacturer instructions. Our results showed that in the Pre-Covid 2019 year in 15% of patients an elevation of liver enzymes was found while in the Covid years 2020 and 2021 the amount of patients suffering from liver disorders was higher - 22 % and 23% respectively. Our findings show that men are more affected than women and the group of mature people of working age is the most harmed one. Additionally, among the three enzymes the aminotransferase GGT demonstrate higher values comparing to transaminases ALAT and ASAT. 20 % of examined patients were Covid (+), 8 % - Covid (-) and for 72 % no data are available. Our results showed that 70 % of Covid (+) patients displayed increased transaminases and only 30 % were not affected. Interestingly, 30 % of COVID (-) patients showed also increased liver enzymes. In conclusion, 70 % of Covid-infected Bulgarian citizens suffer from liver injury due to a dual reason - disease etiology and heavy load of drugs intake during the curing period. The origin of increased liver enzymes in the healthy patients is still unclear and could be a consequence of several reasons - enormous intake of un-prescribed drugs, hepatitis A, B or C, bile duct damage, non-alcoholic fatty liver disease, alcohol abuse, autoimmune hepatitis, hemochromatosis, obesity, toxic hepatitis, toxic spoiled foods consumption, celiac disease, Epstain-Barr virus, pesticide rich foods feeding, sepsis, Wilson's disease, undiagnosed cirrhosis or liver cancer.

Keywords: ASAT, ALAT, GGT, liver damage, Pre-COVID and COVID period

Food Microbiology

FM1

YEAST PROBIOTICS IN RUMINANT NUTRITION AND HEALTH: A REVIEW

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Yeast cultures have been used for a long time in ruminant nutrition. The information on the effects of different yeast species on ruminal microbial population and fermentation is limited. As functional foods yeast probiotics has received considerable attention in the last decade. Currently, probiotic yeast strains have been administered with the aim of improving rumen fermentation efficiency by modulating microbial fermentation pathways. The main activity of yeast-based probiotics is the maintenance and reconstitution of the equilibrium of the rumen microbiota which is achieved by various modes of action.

This review mainly focused on the benefits of probiotic yeast supplements on the microbial population and ecosystem in ruminants; improvement of the rumen environment; stimulation of cellulolytic and lactate utilizing microorganisms; rumen fermentation; feed intake and digestibility; volatile fatty acid production; stabilization of ruminal pH; reducing energy and nutrient loses; enhancing feed efficiency; improvement of animal health and performance; reducing the negative environmental impacts of livestock production and etc.

Keywords: yeast probiotics, rumen fermentation, health, productivity

FM2

MODE OF THE BENEFICIAL EFFECTS OF BULGARIAN PROBIOTIC LACTOBACILLI

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Probiotics (*Pro-for and bio-life*) in the Covid-19 pandemic world are a sound base of the new strategy to maintain and restore if needed, the human microbiome. Healthy homeostasis and presence of beneficial microbiota are keys for human health and well-being. Having this in mind, a large screening for lactic acid bacteria (LAB) with probiotic potential was carried out. Our aim was to study the probiotic mechanisms of the beneficial effects of LAB from different habitats. Several newly characterized *Lactobacillus* strains were pre-selected as candidate probiotics as they were supposed to be able to overcome dysbiosis in the gastrointestinal tract (GIT). Thus, high viability in the gut and potential to colonize the intestine were rated as the promising distinction for further LABs characterization in their role as natural bio-protective agents. Production of bacteriocin-like inhibitory substances is also likely to contribute to their beneficial role. A strain –specific broad spectrum of antifungal, antibacterial and/or antiviral activity has been revealed for LABs isolated from traditional Bulgarian yogurt, white-brined cheese and katak. They belong to *Lactobacillus acidophilus group*, *Limosilactobacillus fermentum, Lactiplantibacillus plantarum, Lacticaseibacillus casei,* and *Lactobacillus delbrueckii* subsp. *bulgaricus* species. The promising results for the parabiotic functionality of LABs isolated from breast milk and vaginal swab samples of healthy women have to be pointed, too. The metabolomics analyses revealed production of important for GIT health SCFAs (Short Chain Fatty acids) and molecules neurotransmitters. The lactobacilli-producers play a role in integrity improvement of the epithelial barrier in gut, as factor of human health. The positive modulatory effects of living LAB cells and /or their active parabiotics are not completely elucidated and practically knowledge is highly limited for Bulgarian LAB isolates, especially. Understanding their probiotic activity may permit modulation of the immune system. Thus, additional assessment is needed and is still in progress. Bulgaria is the motherland of the first probiotic, however a limited number of local strains are realized as probiotics. Based on scientifically proven probiotic assessment, well-characterized active LABs may be implemented in new functional foods or beneficial formulas for disease prevention and health promotion.

Key words: Probiotics, Lactic acid bacteria, Parabiotics

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FM3

PATHOGENIC BACTERIA IN MILK PRODUCTS AND THEIR ANTIBIOTIC RESISTANCE

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Cheese plays a significant role in human nutrition, being one of the most popular ready-to-eat foods produced worldwide. Cheese is rich in microbiota and more than 400 species of LAB, Gram (+) and Gram (-) bacteria, yeasts and moulds have been identified in it so far. The rich nutritional content of cheese favours the development of food borne pathogenic bacteria which spoil the product and harm the human health. The aim of our study was to investigate the microbiota in 7 commercially available imported and local full-cream cheese produced from cow, buffalo, sheep and goat milk. Another task of the study was to search for pathogenic bacteria and evaluate their antibiotic resistance. An amount of different bacterial groups in 1 g of cheese was assessed using SPS agar (for Clostridium), MCA agar (for *Staphylococcus*), KEAA (for fecal enterococci), YGS (for yeasts), Candida agar (for *Candida* spp.) and Nutrient broth (for total number of bacteria). Randomly picked isolates were subcultured on Columbia agar with 5% sheep blood; McConkey agar; Sabouraud and BBL Candida CHROMagar. Identification of isolates was carried out using Crystal system, VITEK 2 and API 20 C AUX Candida. Our results revealed that out of 4 imported blue cheese samples 3 covered the EU standards except 1 which was contaminated with fecal enterococci and *E. coli*/coliforms exceeding the standards. All 3 local cheese samples corresponded the standard except yellow cow cheese which was contaminated with staphylococci exceeding the standard values. Pathogenic bacteria isolated from the cheese samples were further identified as *Staphylococcus simulans*, *Enterococcus avium*, *Candida krusei, Candida parapsilosis*, *Cryptococcus neoformans* and *Sthaphylococcus simulans* and investigated for their antibacterial resistance against 15 antibiotics and 6 antifungals according to EUCAST. The mentioned pathogens cause brain abscesses, endocarditis, and bacteremia in humans and even at a small amount they could be harmful especially to immunocompromised consumers, children or pregnant women. Antibiotic resistance tests demonstrated that *Enterococcus* was sensitive to all 15 tested antibiotics, *Staphilococcus* was sensitive to 9 antibiotics and showed intermediate sensitivity to 4of them, *Candida* isolates were sensitive to 4 antifungals and resistant to 2 of them. Our results demonstrate that possible infections caused during cheese consumption could be successfully handled.

Keywords: dairy products, food control, *Clostridium*, *Enterococcus*, *Staphilococcus*, *Candida*

FM4

MICROFLORA OF WATER SAMPLES FROM SOFIA CITY AND SOFIA REGION

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Drinking water is the main resource of the planet. It plays an extremely important role in sustaining life on Earth. Only purified, fresh water can be used for drinking and domestic purposes. Therefore, water conservation is a major global problem, the solution of which is fundamental for present and future generations. Most of water microorganisms are harmless to humans, but others can cause water borne infections and seriously harm health and even lead to death. For this reason, there are modern methods for detection and identification of infection-causing microorganisms, as well as up-to-date methods for processing, disinfection and purification of fresh water, which is used for drinking, household and agricultural needs. Despite these reliable purification methods, it cannot be assumed that the water we use in our daily lives is completely clean, because there is always a risk of secondary contamination during its transportation. The aim of this report is to study the degree of microbial pollution of several public and private water sources in and around Sofia town, as well as to track the change in the amount of indicator microorganisms during the different seasons of the year. The total bacterial count, coli-titer, presence of *Escherichia coli*, sulphite-reducing bacteria (*Clostridium*), bacteria of the genera *Pseudomonas* and *Enterococcus* and fungi were determined according to standards ISO 5667-11:2009, ISO 5667-16:1998 and ISO 6222:2002. Water quality parameters have been monitored from an ecological point of view not only for humans but also for all other living beings. The water samples from various public sources as fountains in the city of Sofia - Bigerova fountain, Rosarium fountain, Lily Lake fountain, Central Banya fountain, are extremely clean and suitable for drinking and household needs all year. The water samples from the catchment in the village of Zheleznitsa, the well in the village of Dolni Lozen and the fountains in a private properties in the village of Dolni Rakovets cannot be used for drinking or domestic needs, only for drip irrigation and agricultural needs, because of presence of *E. coli* and enterococci. A more serious sanitary-microbiological control of the water supply network in the villages of Zheleznitsa and Dolni Rakovets is needed. Temperature changes during the year affect the microflora of the seven water sources in Sofia region. The amount of all indicator microorganisms increases significantly during the warm months. Endo Agar is more selective than Chromocult Coliform Agar, when testing water samples in parallel on both media. Endo Agar is therefore recommended for more selective water analysis.

Keywords: indicator microorganisms, water quality, water safety, selective media

FM5

EFFECTS OF *LACTOBACILLUS PLANTARUM* POST-METABOLITES ON HT-29 INTESTINAL EPITHELIAL CELL MONOLAYER CHARACTERISTICS

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One of the main characteristics of intestinal epithelial cells that ensure their proper functioning is the ability to form a proper monolayer. The integrity of this monolayer is a guarantee for the implementation of the barrier functions and the selective absorption of nutrients in the small intestine. In the present study, we investigated the effect of post-metabolites released into the culture medium by a late exponential phase Lactobacillus plantarum culture, isolated from a katak, on the transepithelial resistance (TER) of intestinal HT-29 epithelial cells, as well as on the organization of their actin cytoskeleton. The application of the tested media was only from the apical side of the cells to mimic the situation in the body. Both in control cells and in those cells cultured in the presence of the bacterial post-metabolites we observed a smooth increase in transepithelial resistance values with the time. Maximum values of TER were reached at day 3 after the addition the components. Despite the similar course of the curves, we observed about 50% higher TER values for the cells cultured in the presence of exponential bacterial culture medium. These higher values are indicative of lower electrical permeability across the epithelial monolayer, i.e. that it is more stable. For the next 3 days we observed stabilization of TER values. This effect of stabilization of the monolayer was temporary and in about 3 to 4 days the TER values of the control and tested cells dropped down, equaling those of the control. It was an indication for adaptation of the cells to the respective post-metabolites in the environment. We think that the effect of stabilization the monolayer is due precisely to components released by the bacterial cells, because in HT-29 cells cultured in the presence of pure MRS medium, a similar effect was not observed – maximum TER values were established about a day earlier, were much slighter, decreasing over time and equaling those of the control.

The formation of a stable layer of intestinal epithelial cells depends on the organization of their actin cytoskeleton. We therefore studied whether it changes when HT-29 cells are cultured in the presence of bacterial post-metabolites. Immunofluorescence analysis of control cells, after staining with phalloidin, showed that actin filaments were organized in the form of clearly visible stress fibrils, as well as right below the cell membrane, where they formed the cell cortex. Bacterial post-metabolites affected this distribution – we observed less stress-fibrils and rearrangement of actin filaments, with compaction under the cell membrane. Our results show a clear effect of bacterial post-metabolites on the characteristics of the intestinal epithelial cell monolayer. But establishing the exact components causing these effects, as well as the molecular mechanisms underlying them, require further research.

Keywords: *Lactobacillus plantarum*, HT-29, TER, actin cytoskeleton

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FM6

PROTEOLYTIC ACTIVITY OF DIFFERENT LACTIC ACID BACTERIA SPECIES CULTIVATED ON MEDIA WITH PLANT PROTEINS

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The aim of the present study was to determinate of the optimal conditions of proteolysis of plant proteins from various sources by strains representing different lactic acid bacteria species: *Lactobacillus delbrueckii* ssp. *Bulgaricus, L. helveticus*, *L. casei*, *L. plantarum*, *L. gasseri*, *L. fermentum*, *L. acidophilus*, *L. delbr. ssp. lactis*, *L. rhamnosus*, *L. pentosus* and *L. brevis*, all maintained in the LBB Culture Collection of LB Bulgaricum PLC, Sofia, Bulgaria. *L. delbrueckii* ssp. *bulgaricus* strains and *L. helveticus* were cultured for 16 hours at 37°C and 42°C in sterile 10% reconstituted skimmed milk (RSM). *L. casei* and *L. plantarum* strains were tested after 16- and 24-hour cultivation at 32°C and 37°C in sterile 10% RSM and sterile 10% RSM with yeast extract. To assess the proteolytic ability of lactic acid bacteria, we focused on the Church (o-phthalaldehyde spectrophotometric) method, as it allows comparison of results obtained with more diverse in nature protein substrates. Strains of the *L. delbr.* ssp. *bulgaricus* and *L. helveticus* have showed the most proteolytic activity using milk protein as substrate. The best results were obtained with 16 h of incubation at 37°C and therefore all further experiments were performed under these conditions. Selected strains from different studied species with high and low proteolysis in milk proteins were further used in a subsequent study with plant protein media. They were cultivated in various types of plant protein-enriched media. For the purpose of our study, pea protein, soy protein, rice protein, hemp protein and chickpea flour were used. The highest degree of proteolysis was observed in media with pea protein, and the most unsuitable media for cultivation were those in which the main components were hemp or rice protein. When combined milk and plant protein, the highest levels of proteolysis were observed in milk medium enriched with pea protein, which is of interest for the application of mixed plant-milk substrates. With regard to plant proteins alone, actively hydrolyzing strains could be selected from the species *L. helveticus, L. casei* and *L. delbr.* ssp. *bulgaricus* while the representatives of *L. plantarum*, *L. gasseri*, *L. pentosus*, *L. brevis* and *L. fermentum* showed extremely low proteolysis in all studied media.

Keywords: Lactic acid bacteria, milk, plant proteins, proteolysis

FM7

CULTIVATION OF PROBIOTIC LACTIC ACID BACTERIA SPECIES WITH DIFFERENT PROTEOLYTIC ACTIVITY INTO MEDIA BASED ON PLANT PROTEINS

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Nowadays consumer awareness towards healthy diets and changing eating habits has created a huge market demand for new foods with health benefits. In this context fermented products, particularly non-dairy beverages are gaining popularity and acceptance because of their functional benefits. New products have been launched based in fruit and cereals and the incorporation of lactic acid bacteria (LAB) in food and beverages is global trend. Fermented foods have important healthy roles in the human diet and studies have demonstrated that regular intake of fermented foods can reduce the incidence of chronic diseases. In the present study the influence of none-milk media based on the plant proteins in the growth of selected probiotic lactic acid bacteria species with high or low proteolytic activity was studied. Proteolytic activity of different lactic acid bacteria species cultivated on media with plant protein were determinated in our previous study. The selected probiotic lactic acid bacteria species with high proteolytic activity - *L. bulgaricus* J24, *L. bulgaricus* b 58/1, *L. casei* C31, *L.helveticus* AB, *L. lactis* and with low proteolytic activity - *L. plantarum* F12/4**,** *L. bulgaricus* b120, *L. bulgaricus* b 67/2, *L. rhamnosus* were included in our study. All strains are maintained in the LBB Culture Collection of LB Bulgaricum PLC, Sofia, Bulgaria. For the preparation of different types of none - milk media as main components rice protein, soy protein, pea protein, hemp protein, chickpea flour and glucose were used. The probiotic lactic acid bacteria were cultivated in various types of plant protein media at different temperatures 43^{o}C, 37^{o}C , with different percent of inoculation - 1% and 5% (for16 hr), 5% and 10% (for 4-8hr) The change in pH, acidity, the number of lactic acid bacteria were measured. The survival of lactic acid bacteria species during freeze-dried process was studied.

Keywords: probiotic, lactic acid bacteria, plant proteins, cultivation

FM8

VIRULENCE POTENTIAL OF ENTEROCOCCI ISOLATES FROM NON-HOSPITAL ORIGIN

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Enterococci as one of the leading nosocomial pathogens represent an important interest, not only in the medical field but in the food industry as well. The genetic information for virulence factors in non-hospital enterococci is still uncomplete. For this reason, this report aims to perform a molecular survey for the presence of virulence factors in 56 isolates from non-hospital sources (cow milk, Bulgarian yogurt, and the intestinal tract of *C. aspersum*). The presence of genes for virulence factors is assessed by PCR analysis for the 8 most common virulence factors (*cylB*, *esp*, *gls24*, *gelE*, *agg*, *psaA*, *ace*, *nucl*), defining the enterococcal potential for human infection. The expressed products of these genes are: transporter of cytolysin (*cylB*), enterococcal surface protein (*esp*), the stress and virulence protein Gls24 (*gls24*), gelatinase (*gelE*), aggregation substance (*agg*), Mn^{2+} transporter (*psaA*), adhesion factor (*ace*) and nuclease (*nucl*). After the analysis, it is established that none of the snail isolates have the written above virulence genes. Positive amplification is observed only in strains, isolated from yogurt and cow milk. It is established that 30% of the milk isolates contain genes for virulence factors. 39% of the yogurt strains have different types of virulence genes. Despite the smaller percentage, in the milk there is a greater number of virulence genes per strain. For example, in the strain PM3 are identified 5 different genes for virulence factors in comparison to yogurt strains that have maximum 2 virulence genes. That can define the milk isolates as more dangerous for the human health. The genes for virulence factors are divided only between 3 different species- *E. faecalis*, *E. faecium* and *E. gallinarum*. As expected and described in literature *E. faecalis* and *E. faecium* represent almost all the isolates containing those genes (43% and 50%). Only one strain of *E. gallinarum* has a gene for virulence factor. It has gene for the Mn^{2+} transporter, earlier described as an adhesin- *psaA*.

Keywords: *Enterococcus spp.,* virulence factors, genetic potential, gene transfer, food

Acknowledgment: This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018".

FM9

ANTIBIOTIC RESISTANCE OF ENTEROCOCCI WITH DIFFERENT ORIGIN

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The modern era of antibiotics started with the discovery of penicillin and since then antibiotics have transformed modern medicine and saved millions of lives. Unfortunately, in recent years bacteria have developed resistance to nearly all antibiotics. This is a reoccurring theme not only in the medical field but in nature isolates as well. Antibiotic resistant enterococci are a major problem in hospital acquired infections and although foodborne enterococcal infections have never been reported, that does not exclude a possible route of transfer of antibiotic resistance. This study assessed the enterococcal resistance of 54 isolates from different origin (intestinal tract of *Cornu aspersum*, cow milk and Bulgarian yogurt). The most strains were isolated from yogurt – 28, followed by strains from snail intestinal tract – 16 and cow milk – 10. The strains belong to 13 different species - *E. durans, E. casseliflavus, E. gallinarum, E. devriesei, E. mundtii, E. pallens, E. gilvus, E. pseudoavium*, *E. maloduratus*, *E. faecium, E. faecalis, E. species T8_110-2* and *E. sp. VRE83 B1_13_2E.* The antibiotic resistance of the strains was assessed by phenotypic and genetic approach. The phenotypic traits were analyzed with disk diffusion tests. The effect of 10 antibiotics (ampicillin/sulbactam- 10/10µg, chloramphenicol- 30µg, streptomycin- 30µg, neomycin- 10µg, gentamicin- 200µg, kanamycin- 30µg, vancomycin- 5µg, tetracycline- 30µg, cefotaxime- 30µg and erythromycin- 15µg) belonging to 7 classes (β-Lactam antibiotics, aminoglycosides, cephalosporins, tetracyclines, chloramphenicols, macrolides and glycopeptides) were analyzed. Most enterococcal strains show resistance to the aminoglycoside group (streptomycin, neomycin, gentamicin, kanamycin). All isolates have formed relatively large zones with the ampicillin/sulbactam combination (≥21mm). All strains from milk and large portion from the others were defined as resistant to cefotaxime due to the lack of zone of antibiotic activity (0mm). According to CLSI standards all strains were susceptible to chloramphenicol. Resistance to erythromycin show 6 strains (5 from yogurt and 1 from gastrointestinal tract of snail). 21 strains were intermediate. 3 strains were resistant (2 from yogurt and 1 from snail), and 7 strains were intermediate to tetracycline. The presence of genes for antibiotic resistance was evaluated by PCR. The analyzed genes express resistance to gentamicin, kanamycin, chloramphenicol, tetracycline, erythromycin, vancomycin, β-lactam antibiotics, quinolones, macrolides. The highest present antibiotic resistant genotypes were found in milk (70%), followed by 39% from the yogurt and 38% from snail. Those genes were more commonly found in *E. faecium* and *E. faecalis* species (29% and 29% respectively from all strains containing genes for antibiotic resistance), compared to *E. casseliflavus* (17%), *E. gallinarum* (8%), *E. devriesei* (4%), *E. mundtii* (4%), *E. pallens* (4%), and *E. maloduratus* (4%). The presence of *tetM* genes corresponds to phenotypically expressed zones of inhibition ≤ 19mm. Only 3 of the isolates which showed zones ≤19mm do not have *tetM* gene.

Keywords: *Enterococcus spp.,* antibiotic resistance, milk, yogurt, *Cornu aspersum*

Acknowledgment: This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018".

FM10

ASSESSMENT OF THE ABILITY OF *STREPTOCOCCUS THERMOPHILUS* STRAINS TO PRODUCE FOLATES

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Folates are known for their biological activities in the organism. They are polyglutamates usualy containing 5 to 7 glutamate residues while folic acid is a monoglutamate and is not a natural phisiologycal form but is used for industrial enrichment of foods and drinks. The most common folate forms naturally presented in food, including milk, are: dihydrofolate (DHF); tetrahydrofolate (THF); 5-methyltetrahydrofolate (5-MTHF); 5- methylene tetrahydrofolate (5-MeTHF); 5-formyltetrahydrofolate (5-FTHF); 10-formyltetrahydrofolate (10-FTHF); 5-10-Methenyltetrahydrofolate. Therefore, the use of folate-producing microorganisms is a more natural alternative to produce foods with increased levels of the vitamin B9. The aim of the present study was to evaluate a large number of *S. thermophilus* strains for their ability to produce folates during fermentation. The method comprises initial treatment of the samples with rat serum to decompose the polygutamate tailes of the folates and as an antioxidants: ascorbic acid and DTT. The mesuremnt of the amount of the different folate forms was executed by UHPLC MS/MS Fourier transformation massspecctrometry. The 54 assesed strains produced different levels of folates (consisting of the sum of the different folate forms). The results are between 76 micrograms per litre up to 219 micrograms per litre. The mainly produced folate form with highest concentration appeared to be the 5-MeTHF and the form with the lowest concentration is the DHF. Two of the evaluated strains showed results with total folate consentration over 200 µg/l.

Keywords: *Streptococcus thermopilus*, folates, massspectometry

Infection Immunology

II1

TARGETING NEUTROPHILS DURING INFLAMMATION

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Neutrophils are innate immune cells involved in early inflammation during infection or injury and in effector mechanisms in late immune responses. They can contribute to the perpetuation of chronic inflammation associated with tissue damage and to organ dysfunction in several acute and chronic inflammatory, autoimmune and degenerative diseases. Taking into account their role in various disease pathogenesis, neutrophils may present an excellent therapeutic target for treatment. Instead of targeting a particular neutrophil effector mechanism or neutrophil apoptosis we propose an approach of fine-tuning neutrophil homeostasis and function by interference with protein acetylation/deacetylation. Sirtuins (Sirt) are key erasers of epigenetic modifications that regulate gene expression via histone deacetylation, and in addition induce post-translational changes of proteins in the cytosol and in a cell type-specific manner. Their activity and expression can be exploited as a tool to modify neutrophils. We activated sirtuin 1 (Sirt 1) pharmacologically using small molecules in various models - chronic joint inflammation induced by zymosan, arthritis induced by passive transfer of anti-collagen antibodies, mild and severe influenza infection and zymosan-induced lung dysfunction. We found that the Sirt1 activator (SRT2183) altered granulopoiesis, cell homeostasis, functions and senescence of neutrophils. Correspondingly, it had a therapeutic effect as demonstrated by significant suppression of clinical symptoms and histological assessment of joint inflammation in two arthritis models – zymosan induced arthritis and collagen-antibody induced arthritis. However, the drug exacerbates severe influenza virus infection and zymosan-induced lung dysfunction indicating that the modulation of neutrophils, in contrast to the chronic state, is not sufficient to limit acute inflammation.

Keywords: neutrophils, inflammation, therapy, sirtuin 1, influenza

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II2

GLOBAL ANALYSIS OF ANTIBODY REPERTOIRES

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Trivial antibody tests resemble searching the log of immunological encounters of an individual with single keywords. A global view of the antibody repertoire would be rather like a map of all or a large part of the antibodies. Repertoire sequencing is the more developed approach but so far it carries no functional information about the individual clones. The only high throughput functional study of the antibodies on an omics scale is based on biopanning (usually using bacteriophages) of large libraries of peptides with whole repertoires followed by next-generation sequencing of the affinity selected particles. Sometimes this approach is called Deep Panning and the resulting library of 105-106 mimotopes – an imprint of the repertoire diversity, is referred to as IgOme. The IgOme has been shown to produce highly informative data e.g., in the study of antibody response in AIDS and Dengue infection. While demonstrating its capacity for summarizing the humoral immune response, these early uses of the IgOme remained in the orbit of the old paradigm. The antibody repertoire instead has at least one compartment which can be used as a biosensor of the internal environment with much wider application. Reading it can provide biomarkers of a wide range of infectious, autoimmune, and malignant diseases. We have demonstrated an algorithm for extracting IgM IgOme profiles-based biomarkers of brain tumors. Furthermore, we probed the IgM of patients with antiphospholipid syndrome to show that the major change in that compartment is the formation of IgM repertoire holes while the IgG in the same patients acquires new pathogenic specificities. In this case, the utility of presenting IgOme data as a sequence graph and the application of graph theory tools was found instrumental. The IgOme mimotope libraries are usually coupled with high throughput binding assays using microarrays of selected peptides (up to 5000 species). We have shown that a set of patient binding data can be used to build a cross-reactivity graph of the peptide ligands used. Using this type of graph, we could identify IgOme profiles differentiating Alzheimer's Disease and frontotemporal dementia and also extract informative profiles of cross-reactivities between tumor-associated antigens serving as a classifier of brain tumors.

In summary, the IgOme studies based on the appropriate informatics tools could provide a whole new range of diagnostic tests and open the possibilities for new theoretical explorations of the complexity of the immune system.

Keywords: antibody repertoire, IgM, biopanning, mimotopes

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PROGRAM AND ABSTRACTS

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