14th Congress of Microbiologists in Bulgaria with International Participation

PROGRAM AND ABSTRACTS

Hisarya, October 10th – 13th, 2018

14th Congress of Microbiologists in Bulgaria with International Participation

Hotel Augusta / Hisarya, Bulgaria

October 10th – 13th, 2018

Congress
is organized by

Bulgarian Society for Microbiology

at Union of Scientists in Bulgaria

The Stephan Angeloff Institute of Microbiology

(Bulgarian Academy of Sciences)

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Congress Sections

General Microbiology (GM)

Applied Microbiology and Microbial Biotechnologies (AMMB)

Medical Microbiology (MM)

Environmental Microbiology (EnvM)

Virology (Vir)

Infectious Immunology(II)

Food Microbiology (FM)

Veterinary Microbiology and Zoonozes (VMZ)

Varia (Var)

General Program

Wednesday, October 10th, 2018

Transport Sofia – Hisarya
9:00 – 12:00

Registration

Onset 12:00

13:30 Opening ceremony

14:00 – 19:00 Congress session

Thursday, October 11th, 2018

8:30 – 17:30 Congress sessions

17:30 – 19:00 Poster session

Friday, October 12th, 2018

8:30 – 18:30 Congress sessions

18:00 – 19:30 Poster session

19:00 - Congress Adjourn

Saturday, October 13th, 2018

Departure to Sofia

Social Events

Wednesday – Friday, October 10th – 14th, 2018

Art Exibition

Prof. Špiro Radulović – Caricatures and Cartoons

Wednesday, October 10th, 2018

19:30 Welcome Reception at Hotel Augusta, Hisarya

Friday, October 12th, 2018

20:00 Congress Gala Dinner at Hotel Augusta, Hisarya

Saturday October 13th, 2018

9:00 Trip to Starosel, Thracian Tumuli and the Starosel Winery

PROGRAM

Wednesday, October 10th, 2018

Augusta Hotel

12:00 Registration
13:30 Congress opening ceremony

Session One

General Microbiology

Chairs: Angel S. Galabov, Bauke Oudega, Stoyanka Stoitsova

Plenary lectures

14:00-14:30 GM1 **Structural microbiology and the structure and future of FEMS**
Bauke Oudega
FEMS President, The Netherlands

14:30-15:00 GM2 **Phage therapy: more than anti-bacterial action**
Andrzej Górski
L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław

15:00-15:30 GM3 **Design and application of antisense oligonucleotides as antibacterial agents**
Robert Penchovski
University of Sofia "St.Kliment Ohridski", Sofia

15:30-16:00 GM4 **The blood microbiome in latent tuberculosis and healthy individuals**
Stefan Panaiotov[1], Vladimir Milanov[2], Maria Nikolova[1], Elena Nikolova[3], Rumen Dimitrov[4], Reni Kalfin[5]
[1]National Center of Infectious and Parasitic Diseases, Sofia, [2]University Hospital for Respiratory Diseases "St. Sofia", Sofia, , [3]Institute of Experimental Morphology, Pathology and Anthropology, Bulgarian Academy of Sciences, Sofia [4]Faculty of Physics, Sofia University, Sofia, Bulgaria, [5]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia

Coffee break 16:00 – 16:30

16:30-17:00 GM5 **Bacterial biofilms - adaptive mode of life**
Stoyanka Stoitsova, Tsvetelina Paunova-Krasteva, Dayana Borisova
Section of Morphology of Microorganisms and Electron Microscopy, Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, BAS

FEMS & MedILS postdocs summer school

17:00-17:15 FEMS **New opportunities and challenges for excellent postdoc microbiologists**

Vaso Taleski
[1]University "Goce Delchev", Shtip
[2]FEMS Director of Events and Internationalization

Oral reports

17:15-17:25 GM7 **Characterization of a sialidase from *Aeromonas caviae* A40/02**

Stephan Engibarov, Rumyana Eneva, Penka Petrova, Vera Kolyovska, Radoslav Abrashev
Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, BAS, Sofia

Applied Microbiology and Microbial Biotechnologies

Plenary lectures

Chairs: Maria Angelova, Stefano Donadio

17:30-18:00 AMMB1 **Discovery of bioactive metabolites at NAICONS**

Stefano Donadio
NAICONS Srl, Milano, Italy
FEMS Secretary General

18:00-18:30 AMMB2 **Health effects of yogurt - past, present and future**

Stefan A. Denev
Department of Biochemistry and Microbiology, Trakia University, Stara Zagora

18:30-19:00 AMMB3 **Metabolomics and its application in biotechnology**

Milen I. Georgiev[1,2]
[1]The Stephan Angeloff Institute of Microbiology, Plovdiv; [2]Center of Plant Systems Biology and Biotechnology, Plovdiv, Bulgaria

Thursday, October 11th, 2018

Session Two

Medical Microbiology

Chairs: Mariana Murdjeva, Lazar Ranin

Plenary lectures

8:30-9:00 MM1 **Changes in antibiotic susceptibility of *Helicobacter pylori* in last ten years**

Lazar Ranin
Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Belgrade

9:00-9:30 MM2 **Microbiological aspects of joint and bone-marrow infections**

Mariana Murdjeva[1], Lubomir Paunov[2], Atanaska Petrova[1]
[1]Department of Microbiology and Immunology, Medical University-Plovdiv and Laboratory of Microbiology, University Hospital "St. George"-Plovdiv
[2]Department of Special Surgery, Medical University-Plovdiv and Second Surgical Clinic, University Hospital "St. George"-Plovdiv

9:30-10:00 MM3 **From Hildegard von Bingen to evidence-based phytotherapy: insight into herbal remedies for bacterial infections**

Alexandra-Maria Năşcuţiu[1,2]
[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest; [2]"Cantacuzino" National Institute for Medical-Military Research and Development, Bucharest

10:00-10:30 MM4 **Evolution of the mechanism of transmission of infection**

William Monev
National Committee of Control of Infection Diseases and Immunoprophylaxis by Ministry of Health, Sofia

Coffee break 10:30 – 11:00

11:00-11:30 Glaxo Smith-Kline presentation **Booster vaccination vs *Bordetella pertussis* infection in growing up persons**

Magdalena Mircheva
Glaxo Smith-Kline

Oral reports

11:30-11:40 MM5 **New drugs for treatment of infections caused by multidrug - resistant Gram-negative bacteria**

Encho Savov[1], K. Kovachka[1], A. Trifonova[1], E. Kioseva[1], T. Strateva[3]
[1]Military Medical Academy, Sofia; [2]MBAL, Samokov; [3]Medical University, Sofia

11:40-11:50 MM6 **Emergence and clonal spread of carbapenem-resistant *Klebsiella pneumoniae* in Bulgarian University Hospital**

Rossitza Vatcheva-Dobrevska[1], L. Zamorano[2], X. Mulet[2], P Stefanova[1] ,V. Dicheva[1], S. Alberti[3], A. Oliver[2]
[1]Microbiology and Virology Department, University Hospital Queen Joanna, Sofia; [2]Microbiology Department, Hospital Son Espases, Palma de Mallorca; [3]University of the Balearic Islands, Palma de Mallorca

11:50-12:00 MM7 **Micellar curcumin improves the antibacterial activity of the alkylphosphocholines erufosine and miltefosine against pathogenic *Staphyloccocus aureus* strains**

Maya Zaharieva[1], Alexander Kroumov[1], Lyudmila Dimitrova[1], Iva Tsvetkova[1], A. Trochopoulos[3], S. M. Konstantinov[3], M. R. Berger[4], M. Momtchilova[5], K. Yoncheva[5], Hristo Najdenski

[1]Department of Infectious Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, [2]Department of Applied Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [3]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, [4]Toxicology and Chemotherapy Unit, German Cancer Research Center, INF581, D-69120 Heidelberg, [5]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University of Sofia

12:00-13:30 Mini symposium Aquachim PLC **From identification to biochemical phenotyping – modern tools for metabolic research**

1. **Microbial identification – approaches to overcome limitations**
2. **Determination of cellular metabolism and sensibility to chemical agents. Potential for development of novel target antimicrobial agents**
3. **Characterization of phenotypes for taxonomical and epidemiological research**
4. **Optimization of microbial lines and conditions for biotech production**

Tanya Rasheva
AQUACHIM PLC

Lunch break 13:30-14:30

Session Three

Environmental Microbiology

Chairs: Galina Satchanska, Mihail Iliev

Plenary lectures

14:30-15:00 EnvM1 Chemolitotrophic bacteria and *archaea* for practical applications

Mihail Iliev[1], Veneta Groudeva[1], Stoyan Groudev[2]
[1]Sofia University "St. Kliment Ohridski", Sofia; [2]University of Mining and Geology "St. Ivan Rilski", Sofia

15:00-15:30 EnvM2 Clean-up of phenol by environmental bacteria biofilms entrapped in PEO cryogels

Galina Satchanska
New Bulgarian University, Sofia

15:30-16:00 EnvM3 Anaerobic digestion of organic wastes - state of the art and some perspectives

Ivan Simeonov, Dencho Denchev, Venelin Hubenov, Elena Chorukova, Snejanka Mihailova, Vasil Lakov
The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia

Coffee break 16:00 – 16:30

Oral reports

16:30-16:40 EnvM4 Microbial biodegradation as a technological approach for utilization of cellulose containing wastes during long-term manned space missions

Hristo Najdenski[1], Veselin Kussovski[1], Venelin Hubenov[1], Lyudmila Dimitrova[1], Yana Gocheva[1], Elena Chorukova[1], Petar Grozdanov[1], Lyudmila Kabaivanova[1], Dencho Denchev[1], Plamen Angelov[2], Ivan SImeonov[1]
[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, [2]Space Research and Thechnology Institute, Bulgarian Academy of Sciences, Sofia

16:40-16:50 EnvM5 Interplay between crayfish host, its epibionts and oomycet pathogen *Aphanomyces astaci*, causative agent of crayfish plague

Ana Bielen[1], Marija Vuk[2], Karla Orlić[2], Lucija Burić[2], Ivana Maguire[2], Jenny Makkonen[3], Tomislav Vladušić[1], Lidija Šver[1], Reno Hrašćan[1], Sandra Hudina[2]
[1]Faculty of Food Technology and Biotechnology, University of Zagreb; [2]Faculty of Science, University of Zagreb; [3]Department of Biology, University of Eastern Finland

16:50-17:00 EnvM6 Evaluation of the risk of damage on drupaceous fruit species as the result of infection with prunus necrotic ringspot virus (PNRV, PNRSV) and prune dwarf virus (PDV)

Antoniy Stoev
ISSAPP "Nikola Pushkarov", Sofia

17:00-17:10 EnvM7 Biological activity of extracts isolated from *Geum urbanum* L.

Lyudmila Dimitrova[1], Maya M. Zaharieva[1], Nedelina Kostadinova[2], Milena Popova[3], Iva Tsvetkova[1], Spiro M. Konstantinov[4], Vassya Bankova[3], Hristo Najdenski[1]
[1]Department of Infectious Microbiology and [2]Section of Mycology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [3]Laboratory Chemistry of Natural Products, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia; [4]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University Sofia

17:10-17:20 EnvM8 Microbiota in atmospheric aerosols during lidar monitoring of densely populated urban area in Sofia

Ralitza Ilieva[1], Michail Iliev[1], Angelova B.1, I. Grigorov[2], G. Kolarov[2], D. Stoyanov[2], I. Nedkov[2], Veneta Groudeva[1]
[1]University of Sofia "St.Kliment Ohridski", Sofia; [2]Institute of Electronics, Bulgarian Academy of Sciences, Sofia

17:20-17:30 EnvM9 Isolation of the bacteria from genus *Geobacter* and application in microbial fuel cell for electricity generation

Ralitza Ilieva[1], Michail Iliev[1], Irena Spasova[2], Stoyan Groudev[2], Plamen Georgiev[2], Angelova B.[1], Veneta Groudeva[1]
[1]University of Sofia "St.Kliment Ohridski", Sofia; [2] University of Mining and geology"St. Ivan Rilski", Sofia

Poster Session One

17:30-19:00

Chairs: Stefan Panayotov, Lyubka Doumanova, Ekaterina Krumova

GM, MM, AMMB

General Microbiology

GM8 **Actin re-arrangements during infection of A549 and MDCK cultured cells with *Pseudomonas aeruginosa* and its T3SS mutants**

Dayana Borisova[1], Tanya Topouzova-Hristova[2], Tsvetelina Paunova-Kraasteva[1], Undine Mechold[3], Stoyanka Stoitsova[1]

[1]Section of Morphology of Microorganisms and Electron Microscopy, Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, BAS; [2]Department of Cytology, Histology and Embryology, Faculty of Biology, Sofia University "St. Kliment Ohridski, [3]Unitéde Biochimiedes Interactios Macromoléculaires, Départementde Biologie Structurale et Chimie, Intitut Pasteur, Paris, France

GM9 **Cell response of antarctic strain *Penicillium chrisogenum* P29 to short term low temperature exposure**

Ekaterina Krumova, Radoslav Abrashev, Nedelina Kostadinova, Galina Stoyancheva, Jeni Miteva-Staleva, Vladislava Dishlijska, boryana Spasova, Maria Angelova

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

GM10 **Whole genome sequence analysis of lab-adapted *Bacillus subtilis* strains implies strain evolution in 40 years**

Meltem Kutnu, Naz Kocabay, İlayda Baydemir, Nazlı H. Erdal, Gülay Özcengiz

Biological Sciences Dept., Middle East Technical University, Ankara

GM11 **Low temperature stress induces age-related protein damages in antarctic *Penicillium* strains**

Jeny Miteva-Staleva, Ekaterina Krumova, Nedelina Kostadinova, Vladislava Dishlyiska, Boryana Spasova, Radoslav Abrashev, Maria Angelova

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

GM12 **Bioinformatics Web-Based server for bacterial genome analyses**

Nikolet Pavlova, Robert Penchovsky

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

GM13 **Control of gene expression by bacterial riboswitches and their application as drug targets**

Lozena A. Otcheva, Katya B. Popova, Nikolet Pavlova, Martina Traykovska, Robert Penchovsky

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

GM14 **Effects of low-temperature stress on the ultrastructural characteristics of Antarctic strain *Penicillium chrisogenum* P29**

Paunova-Krasteva, Ts.[1], Borisova, D.[1], Krumova, E.[2], Stoitsova, S.[1]

[1]Section of Morphology of Microorganisms and Electron Microscopy, Department of General Microbiology; [2]Section of Mycology, The Stephan Angeloff Institute of Microbiology, BAS, Sofia

GM15 **Probing general toxicity of antisense oligonucleotides to bacterial and mammalian cells**

Katya B. Popova, Lozena A. Otcheva, Martina Traykovska and Robert Penchovsky

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

GM16 **Response of proliferating and quiescent *Saccharomyces cerevisiae* cells to menadion and H_2O_2 toxicity**

Anna Tomova, Polya Marinovska, Ventsislava Petrova

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

GM17 **Influence of thin nanostructured films on the development of *E. coli***

Yulia Kutsova, Dragomira Stoyanova, Manoela. Andreeva, Iliana Ivanova
Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia

Medical Microbiology

MM8 **Macrolide resistance and *EMM* type distribution of group A streptococcal isolates from Bulgarian patients**

Muhtarova A., Gergova R., Markovska R., Mitov I.
Department of Medical Microbiology, Faculty of Medicine, Medical University Sofia

MM9 **Prevalence of rheumatogenic and nephritogenic *EMM* genotypes *Streptococcus pyogenes* in Bulgaria**

Gergova R[1], Muhtarova A[1], Markovska R[1], Mihova K[2], Kaneva R[2], Mitov I[1]
[1]Department of Medical Microbiology, Medical University of Sofia; [2]Department of Medical chemistry and Biochmistry, Molecular Medicine Center, Medical University of Sofia

MM10 **Production and bioactivity of exopolysaccharide from *Porphyridium sordidum***

Juliana Ivanova[1], Ivanina Vasileva[1], Biliana Nikolova[2], Severina Semkova[2], Viktoria Pehlivanova[2], Yana Tsoneva[2], Lyudmila Kabaivanova[3]
[1]Department of Experimental Algology, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia; [2]Department of Electroinduced and Adhesive properties, Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia; [3]Department of Applied Microbiology, The "Stephan Angeloff" Institute of Microbiology, BAS, Sofia

MM11 ***Haemophilus influenzae* vaginitis*. Case report***

Gergana Lengerova[1], Tihomir Dermendzhiev[1,2], Zoya Rachkovska[2], Mariana Murdjeva[1,2], Ekaterina Uchikova[3]
[1]Department of Microbiology and Immunology, Faculty of Pharmacy, Medical University, Plovdiv; [2]Laboratory of Microbiology, University Hospital "St. George", Plovdiv; [3]Clinic of Obstetrics and Gynecology, St. George University Hospital, Plovdiv

MM12 **Anti-staphylococcal effect and *in vivo* toxicity of ethyl acetate fraction from aerial parts of *Geum urbanum* L.**

Lyudmila Dimitrova[1], Stanislav Philipov[2], Maya M. Zaharieva[1], Jeni Miteva-Staleva[3], Milena Popova[5], Lilia Tserovska[4], Ekaterina Krumova[3], Galina Zhelezova[4], Spiro M. Konstantinov [6], Vassya Bankova[5], Hristo Najdenski[1]
[1]Department of Infectious Microbiology and [3]Section of Mycology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Laboratory Pathomorphology, Chair "Anatomy, Histology, Pathology and Forensic Medicine" and [4]Department of Biology, Medical Genetics and Microbiology, Medical Faculty, Sofia University "St. Kliment Ohridski"; [5]Laboratory Chemistry of Natural Products, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia;[6]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia

MM13 **Fecal carriage of extended-spectrum β-lactamase-producing *Enterobacteriaceae* among hospitalized patients in two hospitals in Varna and Sofia**

P. Stankova[1], R. Markovska[1], T. Stoeva[2], D. Ivanova[2], D. Dimitrova[2], M. Bozhkova[2], G. Nedelcheva[2], L Boyanova[1], I. Mitov[1]
[1]Medical University of Sofia, Bulgaria, Medical Faculty, Department of Medical Microbiology; [2]Medical University of Varna, Department of Microbiology and Virology; University Multiprofile Hospital for active treatment (UMHAT) "St Marina", Varna

MM14 **Distribution of virulence genes and *MEC A* in *Staphylococcus aureus* isolates from outpatients and hosptalizated patients**

V. M. Tsitou[1], R. Gergova[1], I. Gergova[2], I. Mitov[1]
[1]Department of Medical Microbiology, Medical University, Sofia; [2]Department of Military Epidemiology and Hygiene, Military Medical Academy, Sofia

MM15 **Outbreak of *Serratia marcescens* in a cardiac surgery clininic**

B. Zaharieva[1], A. Kevorkyan[2],T. Dermendzhiev[3], Y. Zahariev[4], R. Vacheva-Dobrevska[5], I. Ivanov[6], M. Murdjeva[3],T. Kantardjiev[7]

[1]Microbiological Laboratory, University Hospital "Kaspela", Plovdiv; [2]Department of Epidemiology and Disaster medicine, Faculty of Public Health, Plovdiv; [3]Department of Microbiology and Immunology, Faculty of Pharmacy, Medical University, Plovdiv; [4]Department of Vascular Surgery, Faculty of Medicine, MedicalUniversity of Plovdiv; [5]Department of Microbiology and Virology, University Hospital "Tsaritsa Yoanna-ISUL", Sofia; [6]National Reference Laboratory "Monitoring and Control of Antimicrobial Resistance", NCIPD, Sofia; [7]National Center for Infectious and Parasitic Diseases, Sofia

MM16 **Protocols for intimin toxin encoding genes differentiation and characterization in *Escherichia coli***

Ivo Sirakov[1], Tanya Strateva[1], Ralitsa Popova[2], Irina Alexandar[3], Biliyana Borisova[4], Ivan Mitov[1]

[1]Medical University-Sofia, Department of Medical Microbiology; [2]Gen Lab, Ltd; [3]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Science; [4]Medical University-Sofia, Faculty of Dental Medicine

MM17 ***Elizabethkingia miricola* recovered from peritoneal cavity**

Yordan Kalchev[1,2], Atanaska Petrova[1,2], Gergana Lengerova[1], Nikolay Krastev[3], Mariana Murdjeva[1,2]

[1]Department of Microbiology and Immunology, Faculty of Pharmacy, Medical University, Plovdiv; [2]Laboratories of Microbiology and Virology, St. George University Hospital, Plovdiv; [3]Clinic of Gastroenterology, St. George University Hospital, Plovdiv

Applied Microbiology and Microbial Biotechnologies

AMMB4 **Secondary metabolites produced by two newly isolated *Trichoderma* strains**

Marinova Badalova[1], Tsvetana Licheva[2], Veronika Koleva[3], Valentin Savov[1 2 3]

[1]Research Organization and Manufacture of Bioproducts-Bulgaria, Sofia; [2]Sofia University ‘’St. Kliment Ohridski”, Sofia; [3]Balkan Plant Science-Bulgaria, Sofia

AMMB5 **Endophytic fungi from *Leucojum aestivum* L. and their ability for transformation of *Amaryllidaceae* alkaloids**

Ekaterina Krumova[1], Nedelina Kostadinova[1], Jeny Miteva-Staleva[1], Maria Angelova[1], Blaga Mutafova[1], Strahil Berkov[2]

[1]The Stephan Angeloff Institute of Microbiology, BAS, Sofia; [2]The institute of Biodiversity and Ecological Investigations, BAS, Sofia

AMMB6 **Copper and silver in synergism against industrial *E. coli* strain**

Dragomira Stoyanova, Iliana Ivanova

Faculty of Biology, Sofia University “St. Kliment Ohridski”, Sofia

AMMB7 **Influence of nitrogen and phosphate sources on growth and biochemical composition of microalgal cultures**

Ivanina Vasileva, Juliana Ivanova

Experimental Algology, Institute of Plant Physiology and Genetics, BAS, Sofia

AMMB8 **Antimicrobial activity of *Bacillus* and *Lactobacillus* strains against phytopathogenic bacteria and micromycetes**

Veronika Koleva[1], Tsvetana Licheva[2], Rositsa Tropcheva[2], Dmitrii Simenovskii[1], Marina Badalova[3], Valentin Savov[1,2,3]

[1]Balkan Plant Science-Bulgaria, Sofia; [2”]St. Kliment Ohridski” Sofia University, Faculty of Biology, Sofia;[3] Romb Ltd., Sofia

AMMB9 ***Bacillus* strains as an effective treatment of mobile forms of phosphorus in Bulgarian soils**

Tsvetana Licheva, Marina Badalova, Rositsa Tropcheva, G. Deleva, K. Chakalov, Valentin Savov

Sofia University St. Kliment Ohridski, Faculty of Biology

AMMB10 Antibacterial effect of hybrid materials, containing pectin and silver against Gram-positive and Gram-negative bacteria

Tsvetelina Angelova[1], Nadezhda Rangelova[2], Nelly Georgieva[1]
[1]Department of Biotechnology, University of Chemical Technology and Metallurgy, Sofia;
[2]Department of Fundamentals of Chemical Technology, University of Chemical Technology and Metallurgy, Sofia

AMMB11 Antibiotic susceptibility of *Lactobacillus plantarum* strains isolated from homemade Bulgarian cheese

Rositsa Tropcheva, Emanuela Lukach
Department of Biotechnology, Faculty of Biology, Sofia University St. Kliment Ohridski, Sofia

Friday, October 12th, 2018

Session Four

Virology

Chairs: Angel S. Galabov, Dijana Škorić

Plenary lectures

8:30-9:00 Vir1 **Hepatıtıs E vırus ınfectıon: Turkey vs. Europe**
A. Arzu Sayıner
Medical Microbiology Department, Dokuz Eylul University, School of Medicine, Izmir

9:00-9:30 Vir2 **Antienteroviral activity of newly synthesized diaryl ethers**
Ivanka Nikolova
The Stephan Angeloff Institute of Microbiology, BAS, Sofia

9:30-10:00 Vir3 **Pathogenic complexes in plants and their silent players**
Dijana Škorić, Silvija Černi and Martina Šeruga Musić
University of Zagreb, Faculty of Science, Department of Biology, Zagreb

10:00-10:30 Vir4 **Gene silencing and enteroviruses**
Nikolay M. Petrov[1], Mariya I. Stoyanova[2], Angel S. Galabov[3]
[1]New Bulgarian University, Sofia; [2]The Institute of Soil Science, Agrotechnologies and Plant Protection "N. Pushkarov", Sofia; [3]The Stephan Angeloff Institute of Microbiology, BAS, Sofia

Coffee break 10:30 – 11:00

Infectious Immunology

Chairs: Anastas D. Pashov, Petya Dimitrova

Plenary lectures

11:00-11:30 II1 **The universe of proteins as seen by IgM antibodies**
Anastas D. Pashov[1], Milena Todorova[1], Velizar Shivarov[2]
[1]The Stephan Angeloff Institute of Microbiology, BAS, Sofia; [2]SOFIAMED University Hospital

11:30-12:00 II2 **Homeostasis of neutrophils in inflammatatory and degenerative joint diseases**
Milena Leseva[1], Kalin Stoyanov[2], Lora Simeonova[3], Luciano Saso[4] and Petya Dimitrova[1]
[1]Department of Immunology, The Stephan Angeloff Institute of Microbiology, Sofia; [2]Faculty of Medicine, Sofia University "St. Kliment Ohridski", Sofia; [3]Deparrment of Virology, The Stephan Angeloff Institute of Microbiology, Sofia; [4]Department of Physiology and Pharmacology "Vittorio Erspamer", Sapienza University of Roma

12:00-13:00	**Mini symposium ELTA 90M**	**Innovations in the microbiology lab - from sampling to getting the result** **Halina Panicharova** Microbiology sales expert

Lunch break 13:00 -14:00

Session Five

Food Microbiology

Chairs: Hristo Najdenski, Stefan Denev

Plenary lectures

14:00-14:30 **FM1** **Climate changes and foodborne pathogens**
Hristo Najdenski
[1]The Stephan Angeloff Institute of Microbiology, BAS, Sofia

14:30-15:00 **FM2** **Metabolic fluctuations in the metabolism of prebiotic oligosaccharides from lactic acid bacteria**
Ilia Iliev, Iskra Ivanova
[1]Department of Biochemistry and Microbiology, Plovdiv University "Paisii Hilendarski", Plovdiv; [2]Department of Microbiology, Sofia University "St. Kliment Ohridski,Sofia

15:00-15:30 **FM3** **Probiotic solution for vaginal candidiasis**
Kiril Perkov[1], Marzena Rys-Panova[2]
[1]ProViotic AD, Sofia;
[2]St. Sofia Hospital, Sofia

15:30-16:00 **GM6** **Aminoglycoside resistance by ribosomal RNA methyltransferases: molecular insight and perspectives**
Gordana Maravic-Vlahovicek
Faculty of Pharmacy and Biochemistry, University of Zagreb

16:00-16:30 **Biocom-Trendafilov presentation** **Fast and reliable methods for microbiological control of surfaces, food and beverages**
Nikolaj Velikov
Biocom-Trendafilov LtD

Coffee break 16:30 – 17:00

Veterinary Microbiology and Zoonoses

Chairs: Rajko Peshev, Konstantin Simeonov

Plenary lectures

17:00-17:30 **VMZ1** **Pest of small ruminants**
Nikolay Massalski[1], Hristo Najdenski[2]
[1]National Diagnostic and Research Veterinary Medical Institute, Sofia; [2]The Stephan Angeloff Institute of Microbiology, BAS, Sofia

17:30-18:00 **VMZ2** **Studies of molecular biological peculiarity of bovine herpesvirus 4 (BHV 4)**
Rajko Peshev[1], Ivo Sirakov[2]
[1]National Diagnostic and Research Veterinary Medical Institute, Sofia;
[2]Medical University, Dept.of Microbiology, Sofia

18:00-18:30 **VMZ3** **20 years epidemiological, epizootical and phylogenetic investigations on tularemia in Bulgaria**

Krastyo Marinov[1], Anetka Trifonova[1], Hristo Najdenski[2]
[1]Military Medical Academy, Sofia; [2]The Stephan Angeloff Institute of Microbiology, BAS, Sofia

Oral report

18:30-18:40 **VMZ4** ***Chlamydia abortus* and *Coxiella burnetii* related abortions in small ruminants in Bulgaria during a five years period (2013-2018)**

Konstantin Simeonov, Mariela Chilingirova
Laboratory for Chlamydial and Rickettsial Diseases, National Diagnostic and Research Veterinary Medical Institute, Sofia

Poster Session Two

18:00 – 19:30

Chairs: Maria Stoyanova, Lilia Ivanova, Petya Orozova

EnvM, FM, Vir, VM

Environmental Microbiology

EnvM10 Occurence and antimicrobial resistance of *Enterococcus* spp. from Bulgarian groundwater sources

Aleksandar Petrov[1], Georgi Beev[2], Todor Stoyanchev[4], Diana Dermendzieva[3], Toncho Dinev[2], Stefan Denev[2]

[1]Department of Health Care, Trakia University, Faculty of Human Medicine, Stara Zagora; [2]Department of Biochemistry, Microbiology & Physics, Faculty of Agriculture, Trakia University, Stara Zagora; [3]Department of Applied Ecology and Animal Hygiene, Faculty of Agriculture, Trakia University, Stara Zagora; [4]Department of Hygiene, Technology and Control of Food Products of Animal Origin, Faculty of Veterinary Medicine, Trakia University, Stara Zagora

EnvM11 Microbial pretreatment of a gold-bearing sulphide

Groudev S.[1], Nicolova M.[1], Spasova I.[1], Georgiev P.[1], Iliev M.[2], Ilieva R.[2], Groudeva V.[2]

[1]University of Mining and geology"St. Ivan Rilski", Sofia; [2]University of Sofia "St.Kliment Ohridski", Sofia

EnvM12 Bioleaching of chalcopyrite concentrate by means of chemolithotrophic microorganisms from different taxonomic groups

Groudev S.[1], Spasova I.[1], Nicolova M.[1], Georgiev P.[1], Iliev M.[2], Ilieva R.[2], Groudeva V.[2]

[1]University of Mining and geology"St. Ivan Rilski", Sofia; [2]University of Sofia "St.Kliment Ohridski", Sofia

EnvM13 Microbial cleaning of polluted waters connected with electricity generation

Spasova I.[1], Groudev S.[1], Nicolcova M.[1], Georgiev P.[1], Iliev M.[2], Ilieva R.[2], Groudeva V[2]

[1]University of Mining and geology"St. Ivan Rilski", Sofia; [2]University of Sofia "St.Kliment Ohridski", Sofia

EnvM14 Mucus from *Cornu aspersum* – a perspective resource of antioxidant substances

Kostadinova N.[1], Dolashka P.[2], Krumova E.[1], L. Velkova[2], A. Dolashki[2], Abrashev R.[1], Miteva-Staleva J.[1], Spasova B.[1], Angelova M.[1]

[1]The Stephan Angeloff Institute for Microbiology, Bulgarian Academy of Sciences, Sofia; [2]Institute of Organic Chemistry with Centrum of Phytochemistry, Bulgarian Academy of Sciences, Sofia

EnvM15 New plant host of *Pseudomonas viridiflava* in Bulgaria

Mariya Stoyanova[1], Liliya Georgieva[2], Nikolay Petrov[3], Nevena Bogatzevska[1]

[1]Agrobioinstitut, Sofia; [2]Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Pushkarov", Sofia; [3]New Bulgarian University, Sofia, Bulgaria

EnvM16 Physiological, biochemical and molecular-genetic characterization of bacterial strains isolated from spring and healing waters in the region of Haskovo

Nedyalka Valcheva-Jekova[1], **Zapryana Denkova**[2], **Radosveta Nikolova**[2], **Rositsa Denkova**[3]

[1]Department of Biochemistry, Microbiology & Physics, Faculty of Agriculture, Trakia University, Stara Zagora; [2]Department of Microbiology, University of Food Technologies, Plovdiv; [3] Department of Biotechnology, Sofia University "St. Kliment Ohridski", Sofia

EnvM17 Phase sensitivity of Bulgarian phytopathogenic xanthomonads isolated from tomato and pepper plants

Yoana Kizheva[1], Venislava Racheva[1], Zoltan Urshev[2], Sergei Ivanov[3], Petia Hristova[1] and Nevena Bogatsevska[4]

[1]Faculty of Biology, Sofia University, "St. Kliment Ohridski", Sofia; [2]LB Bulgaricum Plc., R&D Department, Sofia; [3]Centre of Food Biology, Ltd., Sofia; [4] Institute of Soil Science, Agrotechnologies and Plant Protection 'Nikola Pushkarov', Sofia

EnvM18 A thermophilic bacterium found in Antarctica

Anna Bratchkova, Veneta Ivanova, Adriana Gousterova, Jordan Manasiev, Dilnora Gouliamova, Margarita Stoilova-Disheva, Svetla Danova

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

Food Microbiology

FM4 Isolation and characterisation of lactic acid bacteria from traditional dairy product katak

Dobreva L.[1], Nemska V[2]. and Danova S.[1]

[1]Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [2] Department of Biotechnology, Faculty of Chemical and System Engineering, University of chemical technology and metallurgy, Sofia

FM5 Microbiological evaluation of bottled K-brand water

Manoela Andreeva, Dragomira Stoyanova, Yulia Kutsova, Iliana Ivanova

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

FM6 Detection of *Paenibacillus larvae* spores in honey by conventional PCR and it's potential for American foulbroot control

Nikolina Rusenova[1], Parvan Parvanov[1], Spaska Stanilova[2]

[1]Faculty of Veterinary Medicine, Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Trakia University, Stara Zagora; [2]Faculty of Medicine, Department of Molecular Biology, Trakia University, Stara Zagora

FM7 Wild growing herbs - natural sources for isolation of lactic acid bacteria

Tsvetanka Teneva-Angelova[1], Katerina Georgieva[1,3], Emilia Atanasova[1], Milena T. Nikolova[2], Ina Aneva[2], Dora Beshkova[1]

[1]The Stephan Angeloff Institute of Microbiology, BAS, Department of Applied Microbiology, Laboratory of Applied Biotechnology, Plovdiv; [2]Institute of Biodiversity and Ecosystem Research, BAS, Department of Plant and Fungal Diversity and Resources, Sofia; [3]Medical University, Faculty of Pharmacy, Department of Chemistry and Biochemistry, Plovdiv

FM8 Bad microbiota in full-cream yellow cheese

Tsenova M.[1], Stefanova P.[2], Dicheva V.[2], Vatcheva-Dobrevska R.[2] and G. Satchanska[1]

[1]Biolaboratory, New Bulgarian University, Sofia; [2]University Hospital Queen Joanna-ISUL, Sofia

FM9 Identification of lactobacilli from traditional Bulgarian white-brined cheese

Veronica Nemska[1], Lili Dobreva[2], Nelly Georgieva[1] and Svetla Danova[2]

[1]University of Chemical Technology and Metallurgy, Sofia; [2]The Stephan Angeloff Institute of Microbiology, BAS, Sofia

FM10 Utilization of human breast milk oligosaccharides from lactic acid bacteria strains isolated from neonatal gut microbiota

Daniela Mollova, Tonka Vasileva, Ilia Iliev

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

Virology

Vir4 **Double antiviral combinations applied by consecutive alternating administration against Coxsackievirus B1 infection in mice**

Adelina Stoyanova[1], StefanPhilipov[2], Gerhard Pürstinger[3], Ivanka Nikolova[1], 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]Institute of Pharmacy, University of Innsbruck, Innsbruck

Vir5 **Antiviral activity of double drug combinations with anti-enterovirals versus Coxsackievirus B1**

Adelina Stoyanova[1], StefanPhilipov[2], Gerhard Pürstinger[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]Institute of Pharmacy, University of Innsbruck, Innsbruck

Vir6 **Effect in vitro of double drug combinations against Coxsackievirus B3**

Adelina Stoyanova[1], Lucia Mukova[1], StefanPhilipov[2], Gerhard Pürstinger[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]Institute of Pharmacy, University of Innsbruck, Innsbruck

Vir7 **HBV and HCV incidence among non-Hodgkin's lymphoma patients in Varna region (2013-2016)**

Tsvetelina Kostadinova[1], Liliya Ivanova[2,3], Denitsa Tsaneva-Damyanova[3], Zhivka Stoykova[2,3]

[1]Education and Research Sectors of Medical Laboratory Assistant, Medical College, Medical University, Varna; [2]Laboratory of Clinical Virology-University hospital "St.Marina", Varna; [3]Department of Microbiology and Virology, Medical University, Varna

Vir8 **Investigation of anti-EA (D) IgG in patients with haematological diseases**

Tsvetelina Kostadinova[1], Liliya Ivanova[2,3], Zhivka Stoykova[2,3], Denitsa Tsaneva[3]

[1]Education and Research Sectors of Medical Laboratory Assistant, Medical College, Medical University, Varna; [2]Laboratory of Clinical Virology, University Hospital "St. Marina", Varna; [3]Department of Microbiology and Virology, Medical University, Varna

Veterinary Medicine and Zoonoses

VMZ5 **Effect of *Staphylococcus aureus* infection on the activities of the alcaline phosphatase, aspartate and alanine transferases in the blood plasma of rabbits**

Teodora M. Georgieva, Ivan Penchev

[1]Faculty of Veterinary Medicine, Trakia University, Stara Zagora

VMZ6 **Comparative study of the effect of electrochemically activated water solutions on *Pseudomonas aeruginosa***

Teodora P. Popova[1], Toshka E. Petrova[1], Mila D. Kaleva[2], Stoil D. Karadzhov[3]

[1]University of Forestry, Faculty of Veterinary Medicine, Sofia; [2]Veterinary Clinic UNION VET, Sofia;[3]Bulgarian Association of Activated Water, Sofia

VMZ7 **Preliminary insight into genetic diversity of *Mycobacterium bovis* strains in cattle in Bulgaria**

Violeta Valcheva[1], Tanya Savova-Lalkovska[2], Albena Dimitrova[2], Yosko Petkov[2], Gergana Hadzhieva[2], Hristo Najdenski[1], Magdalena Bonovska[1]

[1]The Stephan Angeloff Institute of Microbiology, BAS, Sofia; [2]National Diagnostic and Research Veterinary Medical Institute, Sofia

VMZ8 **Outbreak of *Aeromonas hydrophila* associated with the parasitic infection *Ichthyophthirius multifiliis* in European catfish (*Silurus glanis*) juveniles**

P. Orozova, V. Krusteva, Sv. Kirova, T. Hubenova, A. Zaikov

National Diagnostic and Research Veterinary Medical Institute, Sofia

VMZ9 **Determination of proteins in bovine and avium PPD tuberculin**

Gergana Hadzhieva
Department of Research and Development Activities, Biopharm Engineering, Sliven

VMZ10 **Prevalence of *Yersinia enterocolitica* in fattening pigs originated from different regions of Bulgaria**

Maya Gatzovska[1], Maya Zaharieva[1], Ludmila Dimitrova[1], Viktoria Teneva[1], Iva Tsvetkova[1], Tanya Dimova[2], Els van Collie[3], Mark Heyndricks[3], Hristo Najdenski[1]
[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences; [2]Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences; [3]Unit "Food Safety", Institute for Agriculture and Fisheries Research (ILVO), Brusselsesteenweg 370, Melle, Belgium

Varia

Var1 **The glyconoside myconoside, extracted from *Haberlea rhodopensis* Is a promising agent for phytopharmacological development of balanced cytotoxicity**

Ralitsa Veleva[1], Aleksandrina Nesheva[1], Tanya Topouzova-Hristova[1], Jeny Miteva-Staleva[3], Ekaterina Krumova[3], Daniela Moyankova[4], Dimitar Djilianov[4], Aneliya Kostadinova[2]
[1]Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia; [2]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia; [3]Institute of Microbiology, Bulgarian Academy of Sciences, Sofia; [4]AgrobioInstitute, Agricultural Academy, Sofia

19:00 - Congress Adjourn

Saturday, October 13th, 2018

10:00 Trip to Starocel

Thracian Tumuli and the Starosel Winery

Departure to Sofia

ABSTRACT BOOK

General Microbiology

GM1

STRUCTURAL MICROBIOLOGY AND THE STRUCTURE AND FUTURE OF FEMS

Bauke Oudega

FEMS President, The Netherlands

GM2

PHAGE THERAPY: MORE THAN ANTI-BACTERIAL ACTION

Andrzej Górski

Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław

Anti-microbial resistance (AMR) has been recognized as a fundamental threat to human health and one of the greatest challenges to our civilization; it is estimated that by 2050 ten million people may be dying annually and the economic burden may hit $100 trillion. This crisis has greatly revived interest in bacteriophages (phages), viruses of bacteria, as a potential weapon against AMR which could be used to treat patients with infections caused by antibiotic-resistant bacteria. More than ten reviews addressing different aspects of phage therapy (PT) have been published in the past year; recently, *The Lancet* , *JAMA* and *Science* discussed the progress in PT. This well illustrates the growing interest in the potential of PT in parallel to an increase in the threat of AMR. In the past year significant progress in the application of PT in broader clinical practice was noted when successful intravenous application of phages in individual patients in Belgium and USA was described.

While the real therapeutic value of PT still awaits confirmation by clinical trials, the progress in research on immunobiology of phages has supplied new and exciting data which confirm our notion that PT has “potential for evolving from merely a treatment for complications to targeting diseases” (1). Thus, in addition to eliminating bacteria PT might also be considered to control inflammation including that occurring in the course of some non-communicable diseases. These anti-inflammatory and immunomodulating effects of phages have recently been discussed by us in detail (2).

Furthermore, there has been a significant broadening in our knowledge of phage presence within the human body and possible significance of this phenomenon in health and disease. Phages are present in high concentrations in the intestinal tract and can often be detected in ascitic fluid, urine and saliva. Progress in metagenomic analysis has also provided data to indicate that phages are not only present in oropharyngeal and urinary samples but may also circulate in blood.

In the past years our current understanding of the role of phages in nature and their potential in medicine has shifted from mere “viruses of bacteria” that influence the number and functions of bacteria and could be used to combat bacterial infections to the notion of an important component of our organisms abundantly present in the intestines, from where phages can migrate to blood and tissues. Thus, it is becoming evident that phages can interact not only with bacteria but also with eukaryotic cells. Research on the significance of such phage-eukaryotic cell interactions is at a very early stage. However, data obtained so far strongly suggest that phages may induce immunomodulatory effects which can be used in the clinic in PT. Moreover, these novel findings highlight the potential protective role of “endogenous” phages present in the human body (“natural PT”). In this sense, a better understanding of mechanisms responsible for prophage induction *in vivo* and the significance of such phages may yield new and exciting data, including the possible role of hydrogen peroxide in regulating this process in health and disease (Górski et al., Phage therapy: beyond antibacterial action. Front Med doi 10.3389/fmed.2018.00146).

1. Górski A. et al. Phage therapy: combating infections with potential for evolving from merely a treatment for complications to targeting diseases. Front Microbiol 2016,7,1515.
2. Górski A. et al. Phages and immunomodulation. Future Microbiol 2017,12,905-14.

GM3

DESIGN AND APPLICATION OF ANTISENSE OLIGONUCLEOTIDES AS ANTIBACTERIAL AGENTS

Robert Penchovski

University of Sofia “St.Kliment Ohridski”, Sofia

In the last decade, antibacterial drug resistance has emerged as a major challenge in modern medicine due to the rise of many bacterial pathogenic strains that are resistant to all known antibiotics. Here we present the design and applications of antisense oligonucleotides (ASOs) as novel antibacterial agents that target specific bacterial mRNAs. The ASOs are coupled with cell penetrating oligopeptides that deliver them into the cell. These mRNAs are responsible for the function of four different biosynthetic pathways in bacteria that synthesize essential metabolites. We demonstrate a growth inhibition of various pathogenic bacteria, including Staphylococcus aureus, *Listeria monocytogenes, and Escherichia coli by our ASOs. Our approach is very promising since we have achieved 100% efficiency of the bacteria growth inhibition by our designer ASOs.* We believe that our approach for engineering novel synthetic antibacterial agents based on ASOs is applicable to the rapid development of novel classes of antibiotics. This research is supported by a grant, number DN13/14/20.12.2017 awarded by the Bulgarian National Science Fund.

Keywords*:* bacterial pathogenic strains, antisense oligonucleotides (ASOs), novel antibacterial agents

GM4

THE BLOOD MICROBIOME IN LATENT TUBERCULOSIS AND HEALTHY INDIVIDUALS

Stefan Panaiotov[1], Vladimir Milanov[2], Maria Nikolova[1], Elena Nikolova[3], Rumen Dimitrov[4] Reni Kalfin[5]

[1]National Center of Infectious and Parasitic Diseases, Sofia,
[2]University Hospital for Respiratory Diseases "St. Sofia", Sofia
[3]Institute of Experimental Morphology, Pathology and Anthropology, Bulgarian Academy of Sciences, Sofia
[4]Faculty of Physics, Sofia University, Sofia, Bulgaria
[5]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia

Background: A third of the world's population has latent infection with *Mycobacterium tuberculosis*. The tuberculosis reservoir in areas of low endemicity are individuals with latent TB. The factors and moment of reactivation of latent bacilli remain unknown. Little is known about the location of the bacilli in latently infected individuals. Long-term mycobacterial persistence in the lungs has been reported, however persistence in the blood has not been investigated. Hypothesis for the existence of latent *M. tuberculosis* in the blood have been arisen and detection of *M. tuberculosis* in peripheral blood as a diagnostic tool has been applied.

The aim of our study was to target *M. tuberculosis* in the blood of patients with active and latent tuberculosis by blood culturing resuscitation and NGS DNA sequencing. **Results:** Twenty eight blood samples of healthy individuals, 16 of patients with active TB, 4 with latent TB and 10 with other diseases were studied. Several culture media were tested. Blood microbiota resuscitation was performed in BHI broth supplemented with vitamin K 1 mg/ml, 2% sucrose, 0.25% sodium citrate and 0.2% yeastolate at 43^0C for 72 h. All tested blood samples were culture positive, as confirmed by Gram staining and TEM. TEM images demonstrated well defined cell structures. Analysis for bacterial and eukaryotic species was performed by 16S rRNA and ITS2 targeted sequencing. The obtained sequences were clustered (≥97% identity) in Operational Taxonomic Units (OTUs). Among cultured and uncultured samples we identified OTUs similarity with 47 bacterial orders, belonging to 15 phyla (including family *Mycobacteriaceae*) and 39 fungi orders belonging to 2 phyla. Blood-group differences were identified among the bacterial microbiome composition. The preliminary results of the on-going experiments demonstrate that monitoring the dynamics of the blood microbiome could predict latent TB reactivation.

Conclusion: Rich blood microbiome biodiversity is innate of the healthy individuals. Optimization of resuscitation *M. tuberculosis* culture media and conditions are on-going. Interventional strategies to bind the host blood latent *M. tuberculosis* with the states of health and disease are a research goal.

GM5

BACTERIAL BIOFILMS - ADAPTIVE MODE OF LIFE

Stoyanka Stoitsova, Tsvetelina Paunova-Kraasteva,Dayana Borisova

Section of Morphology of Microorganisms and Electron Microscopy, Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

Aim. As an adaptation to hostile environmental conditions - nutrient limitation, inappropriate physicochemical conditions, presence of toxic or antibacterial substances, etc., bacteria attach to surfaces and form complicated multicellular consortia – biofilms. The aim of this report is to present our experience in the studies of bacterial biofilms.

Methods. Qualitative: crystal violet assay, lectin-peroxidase; microscopic: SEM, CLSM.

Results. We characterised the biofilm-forming capacity of different bacterial species and strains. We addressed the response of the attached consortia to treatments against biofilm growth or for biofilms destruction. The need for novel control strategies is justified by biofilm contamination of surfaces in hospitals and food industry, and indwelling medical devices. An obstacle in our efforts to fight biofilms is their wide-range adaptability to hostile conditions. Together with the adaptations of individual bacteria, the structure of biofilms itself, including the extracellular matrix, provides additional defence. The resistance of sessile bacteria to antibiotics is one to several orders of magnitude higher than that of the same strain grown in broth. Biofilm development includes initial attachment, followed by microcolony formation, biofilm maturation and, finally, detachment. The process is influenced by a variety of factors. None of these is indispensable for all species and strains, rather, individual strains use different adaptative mechanisms. Strain- and species-specificity is a problem for control of the attached consortia. We shall present examples of various strain-specific responses to the treatment with antibacterials, probiotic products, plant extracts. Some promising results on the anti-fouling capacity of nanoparticles will also be discussed.

Conclusion. Biofilms are a collective mode of life evolved as an adaptation to environmental stresses. The species- and strain-specific responses to treatments create serious challenges for finding ultimate anti-biofilm solution.

Keywords: biofilm, resistance, strain-specificity

GM6

AMINOGLYCOSIDE RESISTANCE BY RIBOSOMAL RNA METHYLTRANSFERASES: MOLECULAR INSIGHT AND PERSPECTIVES

Gordana Maravic-Vlahovicek

Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia

Bacterial pathogens acquired many resistance mechanisms from natural producers of antibiotics, which protect their cells from the destructive action of their own metabolites. In addition, many of the resistance determinants are variants of different cellular enzymes. Among them are rRNA methylases (MTases), which are responsible for resistance to clinically important macrolide and aminoglycoside that block the protein synthesis. In contrast to mutations in rRNA or ribosomal proteins that emerge only in a portion of genes, the methylation of ribosomal RNA is a particularly efficient mechanism, since it modifies all rRNA copies and usually generates high level of resistance.

Ribosomal 30S subunit is a target for aminoglycoside antibiotics. Many different resistance mechanisms to these antibiotics have been described in clinical strains, but until recently the methylation of rRNA was not among them. In the last decade, however, new types of highly aminoglycoside resistant pathogens have emerged that carry Arm and Kam MTases, specific for G1405 and A1408, respectively, which were previously restricted exclusively to antibiotic producers. Several genes have recently been isolated from plasmids that are now rapidly spreading by horizontal transfer, thus representing a threat to successful treatment of bacterial infections.

Our recent studies on the mechanisms of resistance to aminoglycoside antibiotics based on the action of aminoglycoside resistance MTases will be presented and discussed. Our latest results on the sequence-structure-function relationship will be put in the perspective, with the consideration to find a way to overcome this type of bacterial resistance.

Keywords: aminoglycoside resistance, rRNA methyltransferases, mechanism of action

GM7

CHARACTERIZATION OF A SIALIDASE FROM *AEROMONAS CAVIAE* A40/02

Stephan Engibarov, Rumyana Eneva, Penka Petrova, Vera Kolyovska, Radoslav Abrashev

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

Aim. The study aims to define the molecular weight, pH and temperature optima of a purified sialidase from *Aeromonas* strain, which is identified as *Aeromonas caviae* by PCR assay and ampicillin resistance.

Methods. A 16S rDNA-based PCR was performed to achieve species identification of the strain A40/02 and the ribosomal gene sequence was compared to type strains. The susceptibility of the strain to antibiotics was established by the Bauer-Kirby disk-diffusion method. Native PAGE and SDS-PAGE were applied to evaluate the electrophoretic purity of the enzyme and the molecular weight was estimated using the R_f-factor. In order to determine the pH-optimum, the enzyme assay was carried out in different buffers having a pH range of 2.0 – 10.0. The temperature dependent studies were conducted in the range of 10-80°C.

Results. The comparison of 16S rDNA sequence of A40/02 with corresponding sequences of type strains shows 99.7851% similarity with *Aeromonas caviae* ATCC 15468 and 99.7135% with *Aeromonas enteropelogenes* ATCC 49803. The resistance of the A40/02 strain to ampicillin unambiguously identifies it as *Aeromonas caviae.* The results for molecular weight obtained from native PAGE (130 kDa) and SDS-PAGE (65kDa) suggest a dimeric structure of the sialidase. There is an unusual pH profile with one peak of activity around pH-3.0 and a second broader peak at about pH-7.0. The optimum temperature for activity was found to be 50°C.

Conclusion. The purified enzyme is characterized by some specific properties, such as dimeric structure and pH-optimum with two peaks. Although derived from mesophilic producer, the sialidase demonstrates maximal enzyme activity at 50°C. The study also presents the first report of *Aeromonas caviae* to produce sialidase.

Keywords: *Aeromonas caviae*, sialidase, dimer, temperature optimum, pH optimum

GM8

ACTIN RE-ARRANGEMENTS DURING INFECTION OF A549 AND MDCK CULTURED CELLS WITH *PSEUDOMONAS AERUGINOSA* AND ITS T3SS MUTANTS

Dayana Borisova[1], Tanya Topouzova-Hristova[2], Tsvetelina Paunova-Kraasteva[1], Undine Mechold[3], Stoyanka Stoitsova[1]

[1]Department of Cytology, Histology and Embryology, Faculty of Biology, Sofia University "St. Kliment Ohridski";
[2]Section of Morphology of Microorganisms and Electron Microscopy, Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences
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Aim: The study aims to trace in dynamics, within a 24-hour interval, the structure of the actin cytoskeleton of A549 and MDCK cultured cells harboring intracellular *P. aeruginosa* PA01F+ and its isogenic T3SS mutants.

Methods: The strains used in the study are *P. aeruginosa* PA01 F+ and its T3SS isogenic mutants dY, dTS and dTSY. Confluent monolayers of A549 or MDCK cells were co-cultivated with 10^5 bacteria/ml, then the bacteria were removed and DMEM containing 100 µg/ml gentamycin was added to kill extracellular bacteria. The cells were cultured for further 2, 5, or 23 hours. The structure of the actin cytoskeleton was examined by fluorescence microscopy after staining with TRITC-phalloidin.

Results: The results show actin re-arrangements in the cultured cells that progress with time. The strongest alterations in both eukartyote models were registered on hour 6, with a trend of normalization on hour 24. Comparing the effect of the strains, the cultured cells treated with the triple mutant dTSY were least affected and were closest to the non-infected control cells. Apparently, the dTS mutant that produces ExoY but not ExoT and ExoS causes the highest degree of alteration of the actin cytoskeleton in comparison with the wild-type and the dY mutant.

Conclusion: The T3SS effector toxins alone and in co-operation cause actin re-organisation in the tested models of cultured cells. ExoY alone, in the absence of Exo T and ExoS evokes highest alterations of the microfilament organization. The observed trend for normalization of the actin cytoskeleton on hour 24 indicates some mutual adaptation of the cultured eukaryotes and the intracellular bacteria.

Keywords: A549, MDCK, *P. aeruginosa*, actin re-arrangements

Acknowledgements: The study was supported by PTR 43-16, 2017

GM9

CELL RESPONSE OF ANTARCTIC STRAIN *PENICILLIUM CHRISOGENUM* P29 TO SHORT TERM LOW TEMPERATURE EXPOSURE

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Aim. In this study, Antarctic strain *Penicillium chrisogenum* p29 was tested for the effect of short term exposure to low temperature on a range of physiological parameters and antioxidant enzyme defense. The experiments were designed to obtain information about cell response of the strain isolated from extreme environment.

Methods. The fungal strains, *P. chrisogenum* p29, was isolated from soil samples collected in Livingston Island (Maritime Antarctica) during the Bulgarian Antarctic expedition. To get data about the cell response against short term temperature downshift, exponentially growth-phase cultures were exposure to low temperatures (6 and 15ºC) for 6 hours. After that the temperature was shifted up to the optimal value. Fungal growth, glucose consumption, level of reserve carbohydrates, activity of superoxide dismutase (SOD) and catalase (CAT) were measured.

Results. The low temperature stress had no sizeable effect on fungal growth. Stress caused by 6 hours exposure of the model strain at temperature 6ºC led to delay of glucose consumption in comparison to the cultivation at optimal growth temperature. Low temperature affected significantly the level of reserve carbohydrates. Trehalose amount raised more than 50% after exposure of 6 hours temperature stress. The highest glycogen level was observed in cells subjected to 6ºC short term stress. The protective role of antioxidant enzymes in stress conditions was confirmed. Activity of the first antioxidant enzyme drastically increased under low temperature stress conditions caused by 6ºC.

Conclusion. Short term exposure to low temperature caused oxidative stress events in fungal cells evidenced by increasing in stress biomarker levels and activated antioxidant enzyme system.

Keywords*:* Antarctic fungi, antioxidant enzymes, low temperature stress

Acknowledgments. This work was supported by the National Scientific Fund of the Ministry of Education and Science, Bulgaria (DN-01/1), which is greatly acknowledged.

GM10

WHOLE GENOME SEQUENCE ANALYSIS OF LAB-ADAPTED *BACILLUS SUBTILIS* STRAINS IMPLIES STRAIN EVOLUTION IN 40 YEARS

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Aim: Microbial WGS studies provide an understanding of the link between genetic variations and phenotypic alterations across the strains of *Bacillus subtilis*, highlighting its significance both as a model and industrial organism[1]. *B. subtilis* is known for producing broad spectrum of antibiotics, including bacilysin as the simplest peptide composed of L-alanine and L-anticapsin. We have been studying the impact of disruption of bacilysin biosynthetic operon on cellular physiology of *B. subtilis*.

Methods: For this aim, we conducted a comparative proteomics study by using two different approaches to compare our lab-adapted strain PY79 and its *bacA*-deleted derivative OGU1 (*bacA::lacZ::erm*) constructed in our laboratory[2]. To make sure that the observed changes at proteome level were not due to genomic alterations of strains over time, we attempted to perform a WGS comparison between (BGSC)-deposited PY79, our extensively lab-adapted PY79 and the derivative of the latter, OGU1.

Results: Although we found 5 distinct base substitutions and 1 deletion when the genome sequence of OGU1 and BGSC PY79 were compared, there were no differences except for *bac* operon between OGU1 and its parental, lab-adapted PY79. Genotypic and phenotypic screening of the differences and similarities between the strains confirmed phase variation postulated for *swrAA/1* gene of swarming behavior.

Conclusion: WGS data revealed genomic diversity among siblings of *B.subtilis* 168 implying a marked influence of consecutive strain transfer under laboratory conditions. Further phenotypic and structural analyses are expected to shed light on the functional effects of aforementioned mutations.

Keywords: *B. subtilis*, WGS, bacilysin, strain evolution

GM11

LOW TEMPERATURE STRESS INDUCES AGE-RELATED PROTEIN DAMAGES IN ANTARCTIC *PENICILLIUM* STRAINS

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Aim. Oxidative Stress Theory of Aging postulates that the accumulation of damage caused by reactive oxygen species (ROS) is the underlying mechanism by which organisms age. These ROS induce the accumulation of oxidatively modified proteins, which may be due to increased protein damage and/or decreased elimination of oxidized protein. The ***aim*** of this work was to evaluate the growth-phase-related changes in oxidative damaged proteins and protease activity in two Antarctic strains belonging to different thermal classes.

Materials and methods. The Antarctic fungal strains, psychrotolerant *Penicillium olsonii* and mesophilic *Penicillium waksmanii* were used. Transient temperature down-shift to 6 or 15°C was applied in the exponential- and stationary-growth cultures. The effect of cold stress was evaluated by the accumulation of carbonylated protein content and the level of extra- and intracellular proteinase activity.

Results. The results demonstrated sharp increase in the oxidative modification of proteins and proteolytic activity during cold stress treatment that subsequently decreased after recovery of the optimal temperature. The cell response depends on the degree of stress, growth phase, and temperature characteristics of the strains. More pronounced effect was established in the mesophilic strain than in psychrotolerant ones.

Conclusion. Cold stress accelerates age-related changes in the cultures of Antarctic fungi.

Keywords: Antarctica, filamentous fungi, oxidative stress, low temperature stress

GM12

BIOINFORMATICS WEB-BASED SERVER FOR BACTERIAL GENOME ANALYSES

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Aim: Here we present 13 applets based on a Web server that is suitable for bioinformatics analyses of bacterial genomes. All applets are freely available at http://penchovsky.atwebpages.com/ . In this poster, we explain how microbiologists can use this bioinformatics programs in their work.

Methods: We use Perl and PHP programming script languages to design Web-based bioinformatics server http://penchovsky.atwebpages.com/

Results: One of the most important applets is called Motif Searcher. It enables microbiologists to find any functional sequence of interest in any sequenced bacterial genome. Some of the other programs include reverse complementary DNA and random DNA/RNA/peptide oligomer generators, a pattern sequence searcher, a DNA restriction cutter, a prokaryotic ORF finder, a random DNA/RNA mutation generator. It also includes calculators of melting temperature (TM) of DNA/DNA, RNA/RNA, and DNA/RNA hybrids, a guide RNA (gRNA) generator for the CRISPR/Cas9 system and an annealing temperature calculator for multiplex PCR.

Conclusions: The Web server presented has very helpful programs for microbiologists that can be applied in their everyday work, particularly in motif search of bacterial genomes. This research is supported by a grant, number DN13/14/20.12.2017 awarded by the Bulgarian National Science Fund.

Keywords: Bioinformatics Web-based Server, applets, sequence analyses.

GM13

CONTROL OF GENE EXPRESSION BY BACTERIAL RIBOSWITCHES AND THEIR APPLICATION AS DRUG TARGETS

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Aim: The main goal of this poster is to present the riboswitches as a gene control elements that regulate the gene expression of over 5% of the bacterial genome. There are two main mechanisms for control of gene expression by riboswitches- termination of transcription and prevention of translation. An additional mechanism for control of gene expression is found only in a glmS riboswitch, which is metabolite-inducible RNA self-cleavage. Therefore, the glmS riboswitch acts as a ribozyme as well.

Methods: Riboswitches are gene control elements found mostly in bacteria. They usually reside in the 5’-Untranslated Region (5’-UTR) of many bacterial mRNAs. They control biosynthetic pathways of many essential cellular metabolites, including Flavin Mononucleotide (FMN), Thiamin pyrophosphate (TPP), glucosamine-6- phosphate, lysine, and many others.

Results: There are 17 different classes of riboswitches found in 42 human pathogenic bacteria. Many of these riboswitches can be used as new targets for the development of new antibiotics.

Conclusions: Riboswitches are found in many pathogenic bacteria. Many different classes of bacterial riboswitches can be used as new targets for the development of novel classes of antibiotics. This research is supported by a grant, number DN13/14/20.12.2017 awarded by the Bulgarian National Science Fund.

Keywords: bacterial riboswitches, gene control elements, new antibacterial drug targets.

GM14

EFFECTS OF LOW-TEMPERATURE STRESS ON THE ULTRASTRUCTURAL CHARACTERISTICS OF ANTARCTIC STRAIN *PENICILLIUM CHRISOGENUM* P29

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Aim: The aim of the study is to explore the effects of low-temperature stress on the fine-structural characteristics of Antarctic strain *Penicillium chrisogenum* P29

Methods: The strain was cultivated in bioreactor at 25^{o}C for 48 hours and then subjected to low temperature stress for 6 hours (at 6 and 15^{o}C). After an incubation of 6 h under cold stress conditions, the temperature was up-shifted to the optimal value. Samples collected on 6th hour of the stress were fixed in 4% cacodylate-bufered glutar aldehyde and 1% OsO_4 and dehydrated in graded ethanol series. The samples were processed for scanning (SEM) and transmission (TEM) electron microscopy.

Results: SEM shows that the low-temperature treatments result in increase of the amount of hyphae that have thicker and rough surfaces. Observations by TEM indicate that mitochondria are the most sensitive organelle to the action of the low-temperature stress. The occurrence of swollen mitochondria with reduced number of cristae is registered in the samples treated with 15^{o}C, and their proportion increases in the samples treated with 6^{o}C. Another target sensitive to the stress is the cell plasma membrane of the fungus. Local expansions of the eisosome-like infolds of the outer membrane are registered with the 15^{o}C-stress. In the sample treated with 6^{o}C the incidence and dimensions of this phenomenon is significantly higher. Another indication of cell damage is the increased amount of autophagosomes in comparison with the samples cultivated at the optimum temperature of 25^{o}C.

Conclusion: The low-temperature stress provokes the development of pathological processes in the hyphal cells. The ultrastructural alterations are the highest at the lowest temperature of cultivation, 6^{o}C.

Keywords: antarctic strain, low temperature, stress

Acknowledgements: This work was supported by the NCSI of the Ministry of Education and Science, Bulgaria (grant DN-01/1-2016) to which we owe our sincere thanks.

GM15

PROBING GENERAL TOXICITY OF ANTISENSE OLIGONUCLEOTIDES TO BACTERIAL AND MAMMALIAN CELLS

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Aim: Unfortunately, antibacterial drug resistance has emerged as a major problem in modern medicine due to the rise of many pathogenic bacteria that are resistant either to several commonly used or to all known antibiotics. One approach to develop novel antibiotics is to use antisense oligonucleotides (ASO). Such ASOs have to be very specific by targeting predefined mRNA in bacterial cells and must be not toxic to bacterial and mammalian cells in which the target mRNA is not expressed. There we probe the general toxicity of ASOs to bacterial and mammalian cells.

Methods: We use various *in vitro* methods to assess the general toxicity of ASOs attached to cell penetrating peptides to bacterial and mammalian cells.

Results: We show that in therapeutic concentrations ASOs attached to cell penetrating peptides can have no significant general (non-specific) toxicity to bacterial and mammalian cells.

Conclusions: This research is supported by a grant, number DN13/14/20.12.2017 awarded by the Bulgarian National Science Fund.

Keywords: bacterial cells, mammalian cells, antisense oligonucleotides (ASOs), general toxicity.

GM16

RESPONSE OF PROLIFERATING AND QUIESCENT *SACCHAROMYCES CEREVISIAE* CELLS TO MENADION AND H_2O_2 TOXICITY

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Aim: Study the quiescent *Saccharomyces cerevisiae* cells as a novel smart model system for investigation of the basic mechanisms underlying the toxicity of various harmful compounds in animals and humans.

Methods: *S. cerevisiae* BY4741 cells from logarithmic and stationary growth phase were obtained after prolonged cultivation (168 h) in YPD medium. Quiescent cells were separated from the stationary-phase population by percoll density-gradient centrifugation. Spot analysis was used as a screening method for defining the LD_{50} of menadione and H_2O_2 toxicity. The harmful effect of both compounds was further analyzed through determining the survival rate and oxidative stress biomarkers such as protein and lipid oxidation levels, as well as the ROS concentration.

Results: Significant differences have been detected in the sensitivity of quiescent and logarithmic-grown cells to both studied toxic compounds. Quiescent cells were less sensitive to 5 mM H_2O_2 (74 % survival rate) and more susceptible to the cytotoxic action of 100 μM menadione (30 % survival rate). On the other hand the logarithmic grown cells showed superior resistance on menadione (89%) than on H_2O_2 (40%). The intracellular level of ROS, carbonylated proteins and lipid peroxidation correlates with the survival rate and cell cycle stage (logarithmic or G_0) – the highest concentrations were detected in quiescent cells exposed to menadione.

Conclusions: Obtained results suggest that the response of proliferating and quiescent *S. cerevisiae* cells to toxic compounds differs and depends on both cell cycle stage and compound specific mechanism of cytotoxic action.

Key words: *Saccharomyces cerevisiae*, quiescence, toxicity

GM17

INFLUENCE OF THIN NANOSTRUCTURED FILMS ON THE DEVELOPMENT OF *ESCHERICHIA COLI*

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The rapidly evolving resistance of *E. coli* to traditionally used antibiotics makes the research for new antimicrobial materials a serious modern challenge.

New strategies are needed to control bacterial proliferation, and the application of nanomaterials can be a very promising approach in this respect.

Aim: Investigation of antibacterial effect of thin films made of metals and metal oxides by magnetron sputtering on *E.coli*.

Methods:. Thin films TiO_2: Ag: Cu and TiO_2: SiO_2: Ag are obtained by the magnetron sputtering and have different deposition thicknesses. Inhibition in dynamics is determined by the Koch method and the corresponding optical density measurements. The bacteriostatic effect and adaptation of *E. coli* to the tested nanomaterials can be explained by the ability of this bacterial species to activate special transport pumps for the export of nanoparticles or ions from the cell.

Results: Thin film TiO_2: Ag: Cu has a bacteriostatic effect on *E.coli*, whereas the thin film TiO_2: SiO_2: Ag has a bactericidal effect.

Conclusion: As the used industrial strain is more resistant than pathogenic *Escherichia coli*, the use of the investigated films for clinical and medical purposes may be suggested.

Key words: nanomaterials, thin films, method, bacteria*, E.coli*.

Applied Microbiology and Microbial Biotechnologies

AMMB1

DISCOVERY OF BIOACTIVE METABOLITES AT NAICONS

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NAICONS Srl, Milano
FEMS Secretary General

Natural products represent a major source of approved drugs and still play an important role in supplying chemical diversity for applications in human and veterinary medicine, and for agrochemicals. This source of diversity is particularly important for antibiotics, since most of the approved drugs are of microbial origin, or derivatives thereof, while alternative approaches to antibiotic discovery have not proven as productive. In the course of the presentation, I will provide a brief overview on NAICONS' history, its technology platform, its business model and the approaches to discovery new bioactive molecules. I will also report some recent examples on new chemical classes of bioactive molecules and how they were discovered.

AMMB2

HEALTH EFFECTS OF YOGURT - PAST, PRESENT AND FUTURE

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Yogurt has been part on the human diet for thousands of years. It is one of the most popular fermented milk products worldwide and has gained widespread consumer acceptance as a healthy functional food. During that time a number of health benefits have been associated with its consumption. They are mainly attributed to the presents of viable lactic acid bacteria - *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* as well as a wide range of important macro- and micronutrients. Both culture organisms may benefit from the gut environment, and consequently, have an impact on human health.

Yogurt has traditionally been considered a probiotic carrier food with health-promoting effects. Its healthy properties have been attributed to its biological effects, promoted by the intake of living bacteria responsible for milk fermentation. Review of the literature suggests that viable yogurt cultures *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* can modify the intestinal microbiome, which in turn has a profound impact on overall health. Many up to date clinical studies provide more and more evidence on the multiple health effects associated with yogurt consumption.

The health benefits of yogurt discussed are: effects on intestinal microbiota and gut health, constipation and diarrheal diseases; reduction of serum cholesterol, blood pressure, diabetes, cardiovascular diseases, obesity and metabolic syndrome; immune-stimulation by enhancing innate and adaptive immune responses; reduction of symptoms in lactose intolerance (lactose maldigestion), anti-mutagenic and antitumour activityes of yogurt. This paper summarises the evidence for health-promoting effects of yogurt, focusing on recent data and discussing the perspectives.

Keywords: yogurt, Health benefits, *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophiles,*

AMMB3

METABOLOMICS AND ITS APPLICATION IN BIOTECHNOLOGY

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Per definition metabolomics represents a comprehensive holistic approach, comprising of systematic identification and quantification of all metabolites in an organism, at given conditions. The comprehensive analysis of the chemical fingerprints left by metabolic processes started to play nowadays a crucial role in the personalized medicine [1].

Since the rise of the OMICS age several platforms for high throughput analyses of targeted metabolites have been developed accordingly. Nuclear magnetic resonance (NMR) appears very suitable and adequate platform to carry out metabolomics analyses, as it allows simultaneous detection of diverse range of abundant (primary and secondary) metabolites, which opens novel avenues to fully explore the total biochemical machinery of plants. A great advantage of ^{1}H NMR-spectrometry over the other analytical platforms is the possibility for quantification and hence the direct comparison of concentrations of all compounds present in the sample [2, 3].

Some case studies, from author's laboratory, on the application of NMR-based metabolomics concept in natural products research and plant biotechnology [3-8] will be presented and discussed.

Acknowledgements

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AMMB4

SECONDARY METABOLITES PRODUCED BY TWO NEWLY ISOLATED *TRICHODERMA* STRAINS

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Many *Trichoderma* species not only act as biocontrol agents, but also stimulate plant resistance, and plant growth and development resulting in an increase in crop production.

Aim. The aim of the present study is isolation, identification and characterization of phytohormonal synthesis of strains from genus*Trichoderma*.

Methods. Studied strains were identified by molecular techniques such as DNA fingerprinting (Arisan-Atac et al., 1995) and ribosomal DNA internal transcribed spacers (ITS) analysis. The enzymatic potential of newly isolated strains was determined by theAPI ZYM system (BioMerieux, France). To determine the presence of secondary metabolites with phytohormonal naturewas used chromatographic analysis High Performance Liquid Chromatography (HPLC) tandem mass spectrometry.

Results. Newly isolated micromycete strains were identified as *Trichoderma asperellum* JK and *Trichoderma harzianum* MT. According to the API ZYM test both of the strains produced extracellular enzymes such as alkaline and acid phosphatase, phosphohydrolase,leucine aminopeptidase and N-acetyl-p- glucosaminidase.

The results of chromatographic analysisshowed that strains *T. asperellum* JK and *T. harzianum* MT produce Indole-3-propionic acid, thidiazuronand trans-zeatin in high concentrations.

Conclusions. Two strains from genus *Trichoderma* have been isolated and identified. On the basis of the obtained results it can be concluded that the strains synthesizesecondary metabolites with phytohormonal nature which action on the growth of various plant species.

Keywords:*Trichoderma*, isolation, secondary metabolites, chromatographic analysis.

AMMB5

ENDOPHYTIC FUNGI FROM *LEUCOJUM AESTIVUM* L. AND THEIR ABILITY FOR TRANSFORMATION OF *AMARYLLIDACEAE* ALKALOIDS

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The aim of the study was to isolate and identify the endophytic fungi of *Leucojum aestivum* L., collected from Bulgaria, as well as to study their alkaloid transforming activity.

Methods: For fungal strain isolation different parts of *Leucojum aestivum* L. (root, stem and leaves) were used. Isolates were obtained on PDA medium by tissue inoculation culture after surface sterilization method using 70% ethanol as a sterilizing agent. The isolates were cultivated in the same medium at 28°C for 7 days. Pure fungal cultures were identified after morphological characterization as well as on the basis of ITS1 and ITS4 ribosomal gene sequence acquisition and analyses.

Galanthamine was used for testing alkaloid transforming activity of the isolated strains. For this purpose stationary phase cultures were used. The mycelium was transferred aseptically in phosphate buffer (pH 7.8) containing galantamine dissolved in methanol (2% v/v). The final concentration of galantamine was 1mg ml^{-1}. Samples were taken each 24 hours (up to 168) of the transformation reaction and analyzed by GS-MS.

Results: In total, 87 endophytic fungal strains were isolated. Twenty strains were isolated from the bulbs of *L. aestivum*, 27 from the roots, 28 from the stems, and 12 from the leaves. The fungi represented 13 genera (9 families) belonging to phylum *Ascomycota*. The most abundant genera were *Penecillium*, *Aspergillus*, *Talaromyces*, *Trichoderma*, and *Fusarium*. Among the isolated strains, 78 isolates were assigned to a genus and species level.

Some preliminary results from the transformation of galanthamine by the isolated endophytic fungi are shown and discussed.

Conclusion: *Leucojum aestivum* L. is an important source of endophytic fungi. The ability of these fungi for transformation and/or degradation of alkaloids will add important knowledge for understanding endophyte-host relationships in *Amaryllidaceae* plants.

Acknowledgements: Presented investigation is a part from a research project “Role of *Amaryllidaceae* alkaloids in the endophyte-host relationships” DH 01/13 - 17.12.2016, financially supported by the National Scientific Fund of the Ministry of Education and Science, Bulgaria. Leading organization Institute of Biodiversity and Ecological Investigations, BAS. Project supervisor: Prof. Strahil Berkov.

AMMB6

COPPER AND SILVER IN SYNERGISM AGAINST INDUSTRIAL *E. COLI* STRAIN

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Aim. Investigation of the effective metal concentrations on *E.coli* in two organic media.

Methods. Microdilution assay was used for growth evaluation of *E.coli* ATCC 10536 treated with different concentrations of $AgNO_3$, $CuSO_4$, and their combinations. The original LB medium was double diluted to elucidate the effect of the organic content on metal toxicity.

Results. All tested concentrations of CuSO4 have weak or no effect in the original LB broth, but in double diluted medium, growth inhibition was detected till to 9,5 h and 14,5 h for 1,8 mM and 2 mM $CuSO_4$. 5 hours difference in the growth retention was established after treatment with 15 μM $AgNO_3$ in the two tested media with different organic content.

Strong synergistic effect was proved at reduced metal concentrations. The combination of 2 mM $CuSO_4$ and 10 μM $AgNO_3$ leads to 5,5 h retention in *E. coli* growth in original LB media in contrast to single use of the same metal concentrations. Longer lag-phase was established for 1 mM CuSO4 + 1 μM AgNO3 combination in double diluted LB. The bactericidal concentrations were established too: 6 mM $CuSO_4$ and 120 μM $AgNO_3$ when used separately in original medium. In double diluted medium the bactericidal effect is obtained at lower concentrations.

Conclusion. The organic medium content is critical for antibacterial effect and metal toxicity. As the used industrial strain is more resistant than pathogenic *Escherichia coli*, the use of the industrial strain for laboratory testing purposes may be suggested.

Keywords: organic content, metal toxicity, growth inhibition.

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AMMB7

INFLUENCE OF NITROGEN AND PHOSPHATE SOURCES ON GROWTH AND BIOCHEMICAL COMPOSITION OF MICROALGAL CULTURES

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Aim: Even though the interest in algal biotechnology was not very high till the middle of the XX century, this area of study is highly improved nowadays. One of the most important features of the microalgal production is the carbon dioxide supply. It is not only expensive, but also pollutes the atmosphere with excess gasses. In previous studies with green algae in the laboratory of "Experimental algology", some strains showed a good potential for cultivation under lowered supply of carbon dioxide. Unfortunately, in long term cultivation, the absence of carbon dioxide (CO_2) leads to an increase in pH to incompatible with life values. The aim of this research was to create a suitable nutrient media for green and red microalgae, which has to provide them with all the necessary nutrients and in the same time to keep the normal acidity of the culture under lowered supply of CO_2.

Methods: For this study, two different algal species were studied – the newly isolated strain of the green algae *Scenedesmus sp*. BGP and the red algae *Porphyridium cruentum.*

Results: Modifying the existing medium of Setlik (1967) for green algae and Brody & Emerson (1959) for red algae, we managed not only to achieve a significant biomass yield, but also to maintain the biochemical composition balanced.

Conclusions: Being able to grow microalgae in such optimized conditions helps to lower the cost of the production, to preserve the environment and to receive a quality biotechnological product.

Keywords: *Scenedesmus, Porphyridium*, nutrient medium, growth, biochemical composition

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AMMB8

ANTIMICROBIAL ACTIVITY OF *BACILLUS* AND *LACTOBACILLUS* STRAINS AGAINST PHYTOPATHOGENIC BACTERIA AND MICROMYCETES

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Introduction. In the search for new fertilizers from natural resources to replace or to reduce the use of chemical ones, four bacterial strains were selected to be evaluated for their antimicrobial activity.

Aim. In this work four strains from genus *Bacillus* and genus *Lactobacillus* were tested for their antimicrobial activity against phytopathogenic bacteria and micromycetes.

Material and Methods. Strains ML1- *Lactobacillus paracasei* ssp. *paracasei*, ML2 - *Lactobacillus rhamnosus*, PB1 – *Bacillus pumilus* and PD1 – *Bacillus cereus* were screened for their antimicrobial activity against four species: phytopathogenic bacteria *Clavibacter michiganensis* sub*. michiganensis* (isolate of Bulgarian tomatoes) and *Xanthomonas vesicatoria* T1 (isolate of Bulgarian tomatoes) and micromycetes *Aspergillus niger* and *Fusarium proliferatum* (isolate of corn), using agar diffusion method.

Results. The strains ML1 and ML2 demonstrate inhibition against *Clavibacter michiganensis* sub*. michiganensis* (ML1-25mm; ML2-28mm) and *Xanthomonas vesicatoria* (ML1- 21mm, ML2-31mm). Strain PB1 completely suppress the growth of both micromycetes. Strains ML1 and PD1 have a complete inhibition on *Fusarium proliferatum* growth and respectively 40% and 77% inhibitory effect on *Aspergillus niger*.

Conclusions. From the obtained data it can be concluded that studied strains can be applied as a potential components of microbial biofertilizers against phytopathogenic bacteria and micromycetes.

Keywords: *Bacillus, Lactobacillus*, antimicrobial activity

AMMB9

BACILLUS STRAINS AS AN EFFECTIVE TREATMENT OF MOBILE FORMS OF PHOSPHORUS IN BULGARIAN SOILS

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Introduction. Strains of genus *Bacillus* have the potential to increase the availability of phosphorus to plants by dissolution of inorganic phosphate, which favours the growth of plant species and that has an important economic and agricultural impact.

Aim. In this work six strains from genus *Bacillus* were used to treat mobile forms of phosphorus in Bulgarian soils.

Material and Methods. Soils with low phosphate intensity (Leached chernozem) were enriched with poorly soluble phosphorus compounds and inoculated with the tested strains from genus *Bacillus (Bacillus subtilis* T 2*, Bacillus amyloliquefaciens* T 3*, Bacillus subtilis* T 4*, Bacillus subtilis* T 10*, Bacillus thuringiensis* T 17 *and Bacillus cereus* T 18*)*. Tested strains were inoculated in the soil and incubated for 25 days at 28 ^{o}C. After the incubation period, the degradation of phosphorite flour to available phosphorus was examined by the classical method of Egner-Riehm and by extraction with $CaCl_2$.

Results. The strains T 3 and T 18 demonstrated the highest activity on the phosphorus mobility (inoculated at a dose of 2 ml). The treatment with high doses of the microorganisms- T 2, T 17 and T 18 (15 ml) had the strongest positive effect, the mobile phosphorus was increased with approx. 20%.

The method of mild extraction with $CaCl_2$ showed that the inoculation with strain T 10 leads to an increase of the mobile forms of phosphorus – 24,5 times (dose of 2 ml) and 21,5 times (dose of 15 ml) compare with the control.

Conclusions. The studied strains have a positive impact on the increasing of phosphorus mobility in soils with low phosphate intensity treated with hardly degradable phosphors.

Key words: *Bacillus,* leached chernozem, phosphorus

AMMB10

ANTIBACTERIAL EFFECT OF HYBRID MATERIALS, CONTAINING PECTIN AND SILVER AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

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Aim. The aim of the present study is to evaluate the ability of organic/inorganic hybrid materials, containing pectin and different concentrations of silver to inhibit the bacterial growth of reference strains like *Bacillus subtilis* and *Escherichia coli*.

Methods. For preparation of hybrid materials was chosen the sol-gel method. As inorganic component was used tetraethyl orthosilicate and source of silver was $AgNO_3$. The antibacterial activity of materials was tested according to the agar diffusion test and calculation of cell reduction.

Results. It has been experimentally demonstrated that these silver loaded hybrids show a good antibacterial behavior against these types of microorganisms. The results revealed that the increased silver concentration up to 2.5 wt. % Ag leads to formation of bigger inhibition zones and *B. subtilis* strain 3562 was more sensitive to the materials compared to *E. coli* K12. The second method showed significant cell reduction of both strains in presence of higher silver concentrations in the materials.

Conclusions. The synthesized materials showed pronounced inhibitory effect against both type of bacteria which gives the opportunity to be applied in various fields such water purifications, coatings for medical devices and etc.

Keywords: pectin, silver, antibacterial effect

AMMB11

ANTIBIOTIC SUSCEPTIBILITY OF *LACTOBACILLUS PLANTARUM* STRAINS ISOLATED FROM HOMEMADE BULGARIAN CHEESE

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Antibiotics are the most commonly used agents against microbial infections. The problem associated with their application is that the bacteria are highly adaptive microorganisms and are capable to develop resistance to antibiotics. Furthermore, antibiotic's biological activity is non-selective and affects both pathogenic and resident microbiota of the organism. Probiotic bacteria are used to restore the balance of beneficial bacteria in the gastrointestinal and urogenital tract after antibiotic therapy. According to European Food Safety Authority, these beneficial bacteria must not be resistant to certain antibiotics.

Aim. The aim of the present study is to evaluate the antibiotic susceptibility of *Lactobacillus plantarum* strains isolated from traditional homemade Bulgarian cheese and to reveal their probiotic potential.

Methods. An agar-diffusion method was used in order to estimate the activity of 41 antibiotics with different mechanism of action, against six *Lactobacillus planarum* strains. The results were represented by zones of inhibition.

Results. The highest susceptibility of all tested strains is observed in samples with cell wall synthesis inhibitors (carbenicillin, piperacillin and amoxicillin), protein synthesis inhibitors (chloramphenicol), tetracycline and erythromycin. Only one of the six isolates demonstrated resistance to gentamicin, ciprofloxacin and vankomycin.

Conclusions. Many of the lactic acid bacteria have antibiotic resistance genes that can easily be transmitted to pathogenic bacteria by horizontal gene transfer. Thus the antibiotic treatment becomes ineffective. High antibiotic susceptibility, demonstrated by the tested *Lactobacillus planarum* strains is a prerequisite for their potential application as food additives and beneficial health products.

Keywords: antibiotic susceptibility, probiotics, *Lactobacillus plantarum*, Bulgarian cheese.

Medical Microbiology

MM1

CHANGES IN ANTIBIOTIC SUSCEPTIBILITY OF *HELICOBACTER PYLORI* IN LAST TEN YEARS

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After its discovery in 1982., *H.pylori* was widely investigated as very important human pathogen. It colonizes the human gastric mucus layer of approximately half of the world's population, but it can also be isolated from dental plaque of subgingival region in oral cavity. Infection is usually acquired in early childhood and persists for decades if left untreated. The majority of infected persons display a chronic superficial gastritis without clinical symptoms, although their gastric epithelium clearly shows signs of inflammation. Approximately 50% of symptomatic patients develop peptic ulcer disease, lymphoproliferative disorders or gastric cancer.

Non-invasive or invasive techniques can be used in order to set a diagnosis of H.pylori diseases. The group of non-invasive diagnostic tools consists of: urea breath test, stool antigen test and serology. Invasive techniques are based on gastroscopy and gastric biopsy specimens. They can be used for: urease test, direct microscopy, histology, and cultivation procedure. If the presence of H.pylori is proved and if it is acompained with clinical symptoms certain therapy should be included.

The standard empirical triple therapy for *H. pylori* (PPI, clarithromycin and amoxicillin) proposed at the first Maastricht conference on the management of *H. pylori* infection has become widely used throughout the world. Metronidazole is used instead of amoxicillin in patients with a penicillin allergy. Unfortunately, the success rate of first-line treatment has fallen below the recommended 80% in recent years. Non-compliance and the emergence of antibiotic resistant strains of *H. Pylori* are considered to be the major factors contributing to treatment failure.

That is why we decided to isolate *H. pylori* from gastric byoptic material taken from patients with therapeutic failure or friquent recidives, and to test their susceptibility to: amoxycillin, metronidazole, doxycycline, clindamycin, clarithromycin, azithromycin erythromycin, chiprofloxacin and levofloxacin. Susceptibility testing was carried out by disk diffusion method and E-test. Significant resistance levels were detected for amoxicillin and metronidazole. Fortunately, resistance to clarithromycin was detected only in 7.4% of H.pylori isolates.

MM2

MICROBIOLOGICAL ASPECTS OF JOINT AND BONE-MARROW INFECTIONS

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Etiology. Infection in orthopedic surgery is one of the most dreaded complications and is associated with prolonged morbidity, disability and increased mortality. Etiological agents of arthritis and osteomyelitis differ according to patients' age. Staphylococci are predominant isolates in bacterial septic arthritis among elderly >50 years (75% of all causes), and children up to 15 years, as well as in acute hematogenous osteomyelitis whereas gonococci are most frequent causative agents in septic arthritis among sexually active people between 16 and 50 years of age (75%). Haemophilus influenzae is found in 30% out of all isolates in children below 2 years. Gram negative bacteria are rare isolates (<10%). In pelvic post-surgical infections higher incidence of Gram negatives and multiple microbial flora are found in paraplegic patients and patients with neurological defects. Bacterial reactive arthritis is associated with Campylobacter, Yersinia, Salmonella, Shigella and Chlamydia trachomatis. Viral reactive arthritis is a result of previous infection by Hepatitis B virus, Rubella virus, Mumps virus, Alphaviruses or Parvoviruses. Acute post-traumatic and chronic osteomyelitis are with poly-microbial etiology. Salmonella spp. are most common causative agents of osteomyelitis in patients with sickle cell disease

Periprosthetic joint infection. It occurs in 1 to 2 % of joint replacement surgeries and is one of the leading causes of arthroplasty failure. Biofilms play an important role in the pathogenesis of this infection. Overall reported rates of infectious complications after arthroscopy are low (0.01%–0.48% of procedures). Risk factors for their development are use of intra-articular corticosteroids, prolonged tourniquet time, patient's age >50 years, failure to prepare the surgical site again before conversion to arthrotomy, procedure complexity, and a history of previous procedures.

Principles of antimicrobial therapy and prophylaxis in orthopedic surgery. Microbiological examination is crucial. Antimicrobial prophylaxis lowers the rate of postoperative infections. This is important to protect implanted joints from hematogenous infection and bacteremia. A single antibiotic dose ("single shot") is usually sufficient for prophylaxis of these infections. The risk of extended use of antibiotics as prophylaxis can lead to selection of resistant bacterial strains. Etiological therapy of joint and bone infections is usually with intravenous antibiotics. Antimicrobial treatment for up to 6 months may be necessary in chronic osteomyelitis.

Keywords: orthopaedic surgery, periprosthetic joint infection, pre-operative prophylaxis

MM3

FROM HILDEGARD VON BINGEN TO EVIDENCE-BASED PHYTOTHERAPY: INSIGHT INTO HERBAL REMEDIES FOR BACTERIAL INFECTIONS

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Plants represent the oldest source of pharmacotherapy used by mankind. The medical use of plants strongly evolved over time, being integrated in cultural, philosophical, astrological or mystical systems. Four approaches to the use of plants are considered nowadays: magical / shamanic, energetic, functional dynamic and chemical. Several studies have shown correlations between modern herbal use and the claims provided by ancient European authors like the medieval abbess Hildegard von Bingen or the notorious English herbalist Nicholas Culpeper or by traditional systems (Arabic, Indian, Chinese, and Japanese). About 80% of the population living in the developing countries uses nowadays herbal medicine for treatment. In the developed countries, the current "bio" trend of replacing synthetic drugs with phytotherapy has lead to a constant rise of the sales figures of herbal medicines. So far only a few controlled clinical trials of antibacterial herbal medicines have been published, most of them being methodologically weak. Opening new horizons for the exploration of natural healing sources should be done from the perspectives of safety and efficacy. There is an obvious need for standardization of materials, methods and measures for preparation, preservation, presentation and administration of herbal drugs. It is time for the fundamental principles and approaches of the traditional systems to acquire a solid scientific validation. The development of evidence-based phytotherapy will be of much help in the process of evolving from an ideological overestimation of the power of nature to a scientifically proved option for the treatment of many disorders, including infectious diseases.

MM4

EVOLUTION OF THE MECHANISM OF TRANSMISSION OF INFECTION

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Infection diseases are a manifestation of parasitic interrelations between biological species. The mode of transmissions of microorganisms-parasites to people-hosts presents the mechanism of transmission of infection. The present-day human race inherits the infection diseases by his forefathers-humanoides. Parallel with transforms of this, the pathogenic microorganisms adopts to parasitises on the men. Mechanism of transmission of infection is also a product of the evolution of interrelations between microbes and men. Basic biological mark of species homo sapiens is that it acquires a property who the animals not have – the men owns awareness and creates a society. The social, conscious undertakes actions of men species influences on the mechanism of transmissions of infection joint with biological evolutionary factors. The biological sense of mechanism of transmission of infection is to keeps the species of pathogenic microorganisms in the time. According to different properties and needs of pathogenic microorganisms and behavior of men creates a different mechanisms of transmission of infection and their evolution under the influence of natural and social factors, answering again to basic need of species – to save the species. It is discuss the different mechanisms of transmission.

Keywords: Infection diseases, evolution, mechanism of transmission of infection

MM5

NEW DRUGS FOR TREATMENT OF INFECTIONS CAUSED BY MULTIDRUG - RESISTANT GRAM-NEGATIVE BACTERIA

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Multidrug-resistant / MDR/ Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter baumannii are of great concern and often related to the production of extended-spectrum beta-lactamases /ESBL/ and carbapemenase producing bacteria which represent an increasing global threat. The therapy of nosocomial infections due to these MDR Gram-negative bacteria is challenging also because some of the few active new drugs are not available in Bulgaria. In this article we review the opportunities of some drugs / ceftolozane-tazobactam, ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam, cefepim-zidebactam, aztreonam avibactam, cefiderocol/ and their action in vitro on the problematic for hospital infection pathology Gram-negative bacteria. The recommendations to use these new drugs can be estimated from a clinical point of view as an alternative for the treatment of these life-threatening infections but also as an opportunity to give an opinion in this field, while awaiting for more definitive data latter, after we have good practical experience.

Keywords: MDR bacteria, new antimicrobial drugs

MM6

EMERGENCE AND CLONAL SPREAD OF CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* IN BULGARIAN UNIVERSITY HOSPITAL

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Objectives. Carbapenem-resistant *Enterobacteriaceae*, difficult to treat due to the associated multidrug-resistance profiles, emerged in an University hospital from Bulgaria through last three years. The aim of this study was to identify the molecular mechanism of resistance and the clonal spread of the involved strains.

Materials/methods. Between June, 2013 and August , 2015 twenty two carbapenem-resistant *Klebsiella pneumoniae* isolates, each from a different patient, were registered and analyzed .Between 2015-2017 new 26 emerged CRE isolates are included. Clonal relatedness was assessed through PFGE using xbaI as restriction enzyme. Modified Hodge test, colorimetric carbapenem hydrolysis assay and double disk synergy test (DDST, IMP/MER-EDTA) were used for phenotypic characterization, and multiplex PCR (VIM, IMP, KPC, NDM, OXA-48, GES) for the molecular detection of carbapenemases. The expression of porins was analyzed through SDS-PAGE of Outer Membrane proteins (OMPs) followed by Western blot analysis using specific antibodies.

Results. Two different *K. pneumoniae* PFGE clones, designated A (n=13) and B (n=9) were documented.Clone A showed negative results for carbapenemase production through PCR, modified M-22 and was defective in both major porins, OmpK35 and OmpK36, likely explaining carbapenem resistance. On the other hand, clone B showed positive Hodge test, carbapenem hydrolysis assays, MBL DDST and a positive PCR for NDM, identified as NDM-1 through DNA sequencing. Clone A was spread across the neurology ICU, surgical ICU department and pediatric onco-hematology clinic, while clone B was detected in surgery, cardiology ICU and orthopedic departments.

Conclusion. To our knowledge this is the first report of NDM-1 producing *K. pneumoniae* in Bulgaria . Likewise, this is the first report of clonal dissemination of carbapenem resistance linked to the production of CTX-M-22 in a porin-deficient background. Rapid detection , screening and infection control measures are of paramount relevance for preventing the spread of these major resistance threats through high-risk patients.

Keywords: *Klebsiella pneumoniae, NDM-1,* porin-deficient

MM7

MICELLAR CURCUMIN IMPROVES THE ANTIBACTERIAL ACTIVITY OF THE ALKYLPHOSPHOCHOLINES ERUFOSINE AND MILTEFOSINE AGAINST PATHOGENIC *STAPHYLOCCOCUS AUREUS* STRAINS

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Aim. Aim of the present study was to evaluate the antibacterial and biofilm inhibitory effects of erufosine, a third generation alkylphosphocholine (APC) with strong antineoplastic activity, against pathogenic *Staphylococcus aureus* strains and to compare it with the well-known bacteriostatic and bactericidal properties of miltefosine, the prototype of this class anticancer agents. We searched also for synergistic antibacterial combinations between both alkyphosphocholines and curcumin incorporated in copolymeric micelles based on Pluronic P123 or mixture of Pluronic P123 and Pluronic F127 (P123/F127).

Methods. Minimal bactericidal and inhibitory concentrations, redox activity and biofilm reduction were determined by ISO 20776-1:2006(E), CFU enumeration on agar plates, MTT assay and the test of Stepanovic (1), respectively. The redox activity and drug-drug interactions were calculated with the mathematical software MAPLE.

Results. Erufosine showed a moderate bacteriostatic effect in clinically relevant concentrations (50÷60 μM) towards the tested strains and inhibited to higher extend than miltefosine the redox activity of the treated bacteria at minimal inhibitory concentrations. The effect of both drugs was enhanced through combinations with micellar curcumin. Erufosine showed a stronger median biofilm inhibition at lower concentrations (MBIC50=1.87 μM) than miltefosine (MBIC50=6.0 μM) and curcumin (MBIC50=24.84 μM).

Conclusions. The estimated antibacterial activity of erufosine widens the spectrum of its useful pharmacological effects, which is important for its future clinical application. The synergistic and additive effects based on combination ratio 1:1 between the APCs and P123/F127 micellar curcumin towards methicillin resistant staphylococci could be beneficial for the application of both alkylphosphocholines as anticancer agents, since numerous cancer types are accompanied by bacterial infections.

Keywords: micellar curcumin, alkylphosphocholines, antimicrobial activity and biofilm formation, *Staphylococcus aureus*, drug interactions

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MM8

MACROLIDE RESISTANCE AND *EMM* TYPE DISTRIBUTION OF GROUP A STREPTOCOCCAL ISOLATES FROM BULGARIAN PATIENTS

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Group A streptococcus (GAS) - named *Streptococcus pyogenes* is the major etiological agent of tonsillo-pharyngitis and abscesses and also one of the top 10 infectious causes of death worldwide. The macrolides are the onest alternative in their treatment for patients with penicillin allergy.

The aims of this study were to determine the macrolide resistance and *emm* type distribution of GAS strains isolated from Bulgarian patients with different clinical manifestations.

Methods: A total of 56 clinical streptococcal isolates collected in 2017 year were tested by minimal inhibitory concentrations and molecular-genetic methods.

Results: Macrolide-resistant strains were 32% (n=18) of all strains and the percentages of strains possessing the resistance genes were: *mef(A)* in 72% (n=13), *erm(A)* in 11% (n=2), *erm(B)* in 17% (n=3) of them. Sequence analysis of the *emm* gene is the currently accepted “gold standard” methodology for GAS typing and has been applied to all macrolide-resistant isolates in this study. Strains with *mef(A)* were with various *emm* types (*emm*1, 3, 4, 12), without predominant types. The ones *erm(A)*-positive were 12 and 77 *emm* types; the all isolates with *erm(B)* were *emm*28.

In conclusion, prevalence of macrolide resistance in GAS isolates in our region is high and mainly due to *mef(A)* with heterogeneity of GAS *emm* types and there is no evidence for domination of a certain *emm* clone. The notable result is the detection of association between *emm*28 clone and *erm(B)*.

Keywords: Group A streptococcus, *Streptococcus pyogenes*, macrolide resistance, *emm* genotyping

MM9

PREVALENCE OF RHEUMATOGENIC AND NEPHRITOGENIC *EMM* GENOTYPES *STREPTOCOCCUS PYOGENES* IN BULGARIA

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Streptococcus pyogenes (streptococci goup A or GAS) is a causative agent of a wide variety of respiratory tract, skin and soft-tissue infections. Some autoimmune complications can development such as acute glomerulonephritis, rheumatic heart disease, acute rheumatic fever and others after streptococcal infections due to isolates with rheumatogenic or nephritogenic M proteins, resp. *emm* genes types.

The aim of this study was to determine *emm* genotypes that have been detected in Bulgarian clinical GAS isolates and evaluate their relationship with data for autoimmunity competence.

Methods: Genotyping of the *emm* determinants of 108 GAS isolates from various infections was done according to the protocol of the Center for Disease Control and Prevention (CDC).

Results: Fifteen *emm* types were detected and seven dominant *emm* types in more 80% of Bulgarian GAS isolates were determined: *emm*1 at 25%, *emm*3 – 19%, *emm*4 – 11%, *emm*12 - 11%, *emm*2 – 6%, *emm*89 – near 6%. It is important to note that three of the most often types *emm*1,3,12 are known as rheumatogenic and *emm*1,12 and 4 are known as nephritogenic ones.

Conclusions: This is the first report of a rheumatogenic and nephritogenic GAS *emm* types circulating in Bulgaria. It is known that autoimmune complications are associated to a limited number of *emm* types. Our results present variety of *emm* types in the studied *GAS* isolates with prevalence of seven *emm* types. Three among the predominant types were with rheumatogenic ability and three ones of them - with nephritogenic. Their high frequency in our country is alarming.

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MM10

PRODUCTION AND BIOACTIVITY OF EXOPOLYSACCHARIDE FROM *PORPHYRIDIUM SORDIDUM*

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Aim. The aim of the research was to isolate, purify and test the anticarcinogenic properties of exopolysaccharide from the red microalga *Porphyridium sordidum.*

Methods. Intensive cultivation of the microalga was performed at 26℃ and aeration with CO_2- 2%, illumination 8000 lux. Dialysis was applied for purification. Polysaccharide concentrations used were: 10, 25, 50, 75 and 100 µg/ml, applied on $1x10^5$ cells. Two carcinogenic and one normal cell lines were tested in the *in vitro* experiments. MTS test (Promega) was performed for cell survival and proliferation.

Results. Red algae (Rhodophyta) contain several classes of well-known polysaccharides, having wide application in microbiology, biotechnology and other fields. There is little information concerning anticarcinogenic properties of polysaccharides from marine microalgae, although these organisms have been used for a long time as food for humans, particularly *Porphyridium*. Inhibition of cell proliferation of cell lines: low metastatic MDA-MB 231, high metastatic MCF-7 and normal MCF-10A by the isolated from the red microalga *Porphyridium sordidum* exopolysaccharide was estimated in this study. The effect was dose dependent. At concentration 50µg/ml with the high metastatic MCF-7 cell line the registered inhibition was 35% and at 75 µg/ml it was 40%. For the low metastatic MDA-MB 231 cell line the effect of inhibition was less expressed- up to 30%.

Conclusions. The polysaccharide synthesized and released into the surrounding medium by the red unicellular alga showed promising properties as an anticarcinogenic agent. Further studies will help to elucidate the mechanism of action of this bioactive compound.

Keywords: *Porphyridium sordidum*, exopolysaccharide, tumor cells

Acknowledgement: This work was supported by project DN11/2 of the National Scientific Fund of the Ministry of Education and Science, Bulgaria

MM11

HAEMOPHILUS INFLUENZAE VAGINITIS*. CASE REPORT*

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Introduction. *Haemophilus influenzae* (Hi) is an important pathogen which often causes otitis, meningitis, pneumonia, sinuitis and fatal neonatal sepsis associated with premature birth. This micro-organism can be found in the vagina in puberty girls. Following the Hib vaccination, the non-capsular strains infections increase.

Aim. We present a rare case of mixed vaginal infection in a pregnant patient with no accompanying diseases attended the Obstetrics and Gynecology Clinic of the University Hospital "St. George "- Plovdiv.

Material and Methods. Vaginal discharge was cultured on a blood agar, EMB-agar and CHROM agar *Candida*. A lot of small colonies less than 0.5 mm in diameter were found on the primary colonies. They were located around the colonies of another isolate - *Staphylococcus haemolyticus*. Clinical, microbiological (conventional and automated), immunological and molecular-biological diagnostic methods were used. Antibiotic sensitivity was determined by the Bauer-Kirby disk-diffusion method.

Results. Microbiological (MALDI-TOF) and molecular genetic (Multiplex PCR) tests have conclusively demonstrated the presence of *Haemophilus influenzae non-type b* in the vaginal discharge of the patient. The strain was susceptible to penicillins, carbapenems, quinolones and tetracycline.

Conclusion. This mixed vaginal infection in a pregnant women with *Haemophilus influenzae* and *Staphylococcus haemolyticus* was described for the first time in Bulgaria. This case highlights the large etiological diversity of vaginal infection and directs the attention of clinicians and microbiologists towards the search of less common etiological agents as a cause of vaginal symptoms.

Key words: vaginitis, *Haemophilus influenzae*, pregnancy

MM12

ANTI-STAPHYLOCOCCAL EFFECT AND *IN VIVO* TOXICITY OF ETHYL ACETATE FRACTION FROM AERIAL PARTS OF *GEUM URBANUM* L.

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Introduction. The staphylococcal skin and nosocomial infections are often accompanied by production of toxins and biofilm formation in immunocompromised patients.

Aim. We investigated the *in vitro* anti-staphylococcal biofilm effect, the *in vitro* cytotoxicity in human cell lines and the *in vivo* toxicity potential of ethyl acetate (EtOAc) extract from the medicinal plant *Geum urbanum* L.

Methods. We used phytochemical and bioautography methods to select active subfractions from EtOAc fraction, obtained from crude methanol extract, calculated their minimal inhibitory and bactericidal concentrations (MIC/MBC) [ISO 20776-1:2006(E)] and determined the dehydrogenase activity of several pathogenic *Staphylococcus aureus* strains by MTT-test. The *in vitro* cytotoxicity was tested on the cell lines HEK-293 (human normal transformed kidney embryonal cells) and HEP-G2 (human hepatocytes) by MTT [ISO 10993-5] and CFU assays. The acute and subacute toxicity of EtOAc extract were investigated on H-albino mice by hematology study, $^{\bullet}O_2^-$, H_2O_2, SOD, lipid peroxidation and damaged protein in the blood plasma and brain. Histological preparations of liver and kidney were prepared for pathoanatomic analysis.

Results. The EtOAc extract and selected fractions showed significant anti-biofilm potential. Their cytotoxicity varied depending on the fraction, cell line and concentration applied. Slight expressed *in vivo* toxic effects were observed after *per os* administration of the highest concentration – 0.21 mg/kg.

Conclusions. The EtOAc proved effective to concentrate anti-staphylococcal compounds with favorable toxicological profile. The results will contribute to the development of alternative approaches for treatment of skin staphylococcal infections in the medical practice.

Key words: *Geum urbanum,* ethyl acetate extract, anti-staphylococcal biofilm formation, *in vivo* toxicity

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MM13

FECAL CARRIAGE OF EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING *ENTEROBACTERIACEAE* AMONG HOSPITALIZED PATIENTS IN TWO HOSPITALS IN VARNA AND SOFIA

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Gastro-intestinal tract is reported as an important reservoir for ESBL-and carbapenemase-encoding genes, and is associated with one of the major routes for transmission and dissemination of ESBLs and carbapenemase producing bacteria. The aim of this study was to investigate the prevalence of ESBL and carbapenemase producing enteric bacteria from fecal samples, collected from patients, hospitalized in two hospitals in Varna and Sofia (n=311) and hospital personal (n=92).

Materials and methods: During the period January 2014 – September 2014, a total of 100 enterobacterial isolates, resistant to 3rd generation cefolosporins, were isolated from the fecal specimens of 403 patients, hospitalized in ICU and non-ICU wards of University Hospital "Saint Marina", Varna and Second Town hospital - Sofia. Antimicrobial susceptibility testing, PCR and sequencing for detection the presence of beta-lactamase genes were carried out.

Results: the prevalence of 3rd generation cephalosporin resistant isolates was 31% among patients and 4% among hospital personal: *Escherichia coli*, n=70; *Klebsiella pneumoniae*, n=20; *Enterobacter cloacae*, n=6; *Enterobacter aerogenes*, n=3 and *Citrobacter freundii*, n=1. ESBLs production was confirmed for all except for two E. cloacae isolates. The following resistance rates were detected – for amoxicillin/clavulanic acid(55%); ceftazidime(78%); cefotaxime (100%); cefoxitin(13%); cefepime (70%); tobramycin(56%); gentamicin(52%); amikacin(20%); ciprofloxacin(26%);levofloxacin(15%);tetracycline(43%);trimethoprim/sulfamethoxazole(55%); chloramphenicol(25%). Three isolates were non-susceptible to imipenem(3%). The PCR and sequencing revealed the presence of *bla*CTX-M-15 in 43% of isolates, *bla*CTX-M-3 in 38% and *bla*CTX-M-1 and *bla*CTX-M-14 in 1% and 11% respectively. Two isolates were SHV-12 producers.

Concusion: the prevalence of ESBL producing enterobacteria in the gastro-intestinal tract of the studied patients was 19%. A diverse spectrum of ESBLs was identified with CTX-M-15 and CTX-M-3 being the major ESBL types. The emergance of carbapenemase producing bacteria showed necesity for further investigation.

MM14

DISTRIBUTION OF VIRULENCE GENES AND *MEC A* IN *STAPHYLOCOCCUS AUREUS* ISOLATES FROM OUTPATIENTS AND HOSPTALIZATED PATIENTS

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Introduction*. Staphylococcus aureus* is a major cause for both community acquired as well as nosocomial infections, such as soft tissue and bone infections, respiratory tract infections, bacteremia and sepsis*.*

The aim of this study was to determine and evaluate the frequency of genes of twelve virulence factors and *mecA* for methicilin resistance in Bulgarian isolates *S. aureus* from outpatients and hospitalizated ones.

Methods: Clinical isolates (n=218) from 125 inpatients in three University Hospitals in Sofia city and from 93 outpatiens were examined. All biochemically determined *S. aureus* strains were again confirmed by specific polymerase chain reaction (PCR). Staphylococcal strains were screened for the presence of *mecA* and virulence genes using multiplex PCRs.

Results: The distribution of virulence genes from inpatients/outpatients *S. aureus* isolates in percent were: *hlg* (88.00/68.82) > *sei* (77.60/86.02) > *seb* (45.60/76.34 > *seh* (44.8/74.19) > *seg* (44.80/26.88) > *sec* (44.00/10.75) > *sej* (24.00/38.40) > *sed* (20.00/9.68) > *sea* (16.00/18.28) > *see* (15.20/8.60) > *cna (*12.00 /11.83) > *tst-1 (*11.11/1.73) and *mecA* (24.00/12.9).

Conclusion: The genes *hlg, seg, sec, sed, see, tst-1* and *mecA* were more often detected in inpatients isolates, but others such as *sei, seb, seh* were predominant in staphylococcal strains from outpatients. *S. aureus* isolates from inpatients presented more virulence factors and resistance genes than the ones from outpatients ($p<0.05$). The role of enterotoxins is diverse and some correlation with methicillin resistance can established. Staphylococcal TSST-1 and enterotoxins exhibit a super-antigen activity with blocking effect on the immune system that is a key for development of severe infections.

Key words: *Staphylococcus aureus*, virulence, PCR

Acknowledgements: This study was supported by a Grant from the Medical University of Sofia (Council of Medical Science, Project No 8263/2016, Grant No D-56/2017)

MM15

OUTBREAK OF *SERRATIA MARCESCENS* IN A CARDIAC SURGERY CLININIC

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Currently 1-2% of the world's nosocomial infections are caused by *Serratia marcescens,* but its role in nosocomial outbreaks is significant. The aim of the present study was to describe nosocomial outbreak, localized in a cardiac surgery clinic (CSC) of a large university hospital in Bulgaria. The outbreak affected a total of 7 patients for a period of 11 days. Molecular typing (REP-PCR/BOX-PCR and Beta-lactamase screening by Multiplex-PCR) was used to identify the genetic similarity between the isolates of *Serratia marcescens. S. marcescens* with an identical phenotype of antimicrobial resistance and genetic profiles was isolated from all 15 samples from 7 patients - 11 blood cultures, 3 PVC tips cultures and 1 skin swab from area around a PVC and from 2 hospital surfaces - medicine cabinet and sink basin in the nurses' room. We found a common-use IV container with heparinized saline solution, prepared to maintain the patency of PVC for all patients in the clinic, but no microbial contamination of the open IV container was established. The analysis of the microbiological, clinical and epidemiological data from the patients and the hospital environment, points to a localized in-hospital outbreak with a focal source and exogenous transmission appearing in the clinic. The spread of the strain in the hospital environment is due to serious oversights when performing infusion therapy and when decontaminating the surfaces.

MM16

PROTOKOLS FOR INTIMIN TOXIN ENCODING GENES DIFFERENTIATION AND CHARACTERIZATION IN ESCHERICHIA COLI

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Aim. Intimine, as an external membrane protein, is a major virulence factor in enterohaemorrhagic and enteropathogenic Escherichia coli and is associated with adhesion to mammalian host cells. The large variety of intimin toxins is known, with 29 variants identified so far, for which individual pairs of primers were used. In order to reduce the time and cost of identification (by polymerase chain reaction (PCR)) and characterization (by DNA sequencing) of the intimine variants, we set ourselves the goal of developing consensus primers and protocols.

Methods. In the study, we used Sequence data from the NCBI Genbank, E. coli strains isolated from us from food products, gradient PCR and sequencing.

Results. The obtained PCR products of between 1990 and 2478 bp were sequenced and subjected to nucleotide and amino acid analysis, the results of which were confirmed by the specificity of the primers used.

Conclusions. The proposed original primers provide a relatively quick and accessible way of differentiation and study of the intimin production encoding genes.

Keywords: *E. coli,* intimin*,* PCR, sequencing, original protocols.

Acknowledgements: This work was supported by Grant No 8456/09.12.2016; contract № Д-64/02.05.2017 from the Medical University of Sofia, Bulgaria, entitled “Cloning of genes responsible for toxigenicity of Shiga toxin producing *Escherichia coli* isolates and study of their interaction with the cellular receptor CD77”.

MM17

ELIZABETHKINGIA MIRICOLA RECOVERED FROM PERITONEAL CAVITY

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Aim. To focus the attention to a new emerging pathogen that might be associated with infections of the peritoneal cavity and to emphasize the need of precise microbiological analysis.

Material and Methods. A 64-year-old man with liver cirrhosis due to alcohol overuse presented with fatigue, scrotal edema and abdominal swelling. Paracentesis was performed because of the fluid accumulation and a specimen was collected for microbiological analysis. The identification was performed using conventional biochemical tests, API-20NE (BioMerieux), automated Vitek-2 system (BioMerieux), MALDI-TOF (Vitek-MS) and gene sequencing.

Results. Initially the microorganism was misidentified as *Sphingobacterium spiritivorum* for two consecutive times by Vitek-2 system. It was identified twice by API 20 NE as *Elizabethkingia meningoseptica*. MALDI-TOF was run, which has also misidentified the bacterium as *E. meningoseptica* regardless the bacterium is included in the database. The bacterium was identified as *E. miricola* by running conventional biochemical tests and gene sequencing. The isolate was susceptible to fluoroquinolones, trimethoprim/sulfamethoxazole, vancomycin and resistant to aminoglycosides, colistin. Following treatment with levofloxacin the patient was discharged from the hospital.

Conclusions. To the best of our knowledge this is the first case around the globe of *E. miricola* isolated from the peritoneal cavity in patient with ascites. Confirmation of the pathogenic role of this microorganism is challenging but we assume this unusual bacterium might have the potential to cause infection of the peritoneal cavity in immunocompromised hosts.

Key words: *Elizabethkingia miricola*, peritonitis, liver cirrhosis

Environmental Microbiology

EnvM1

CHEMOLITOTROPHIC BACTERIA AND *ARCHAEA* FOR PRACTICAL APPLICATIONS

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Microorganisms are able to interact with metals and minerals in various ways and are involved in global cycling of all elements. One group of microorganisms which especially impacts on the iron and sulphur cycles as well as heavily influencing metal mobility in the geosphere are acidophilic chemolitotrophic bacteria and archaea.

The past and current research and industrial activities in the field of mineral processing and biohydrometalurgy connected with chemolitotrophic microorganisms cover a large number of problems: microbial communities of different mineral deposits, physiology, biochemistry and genetics of the microorganisms, participating in the transformations of the different mineral deposits, bioleaching of copper and other non-ferrous metals and uranium concentrates.

Different techniques for application of metabolic activities of these microorganisms as in situ leaching dump, heap and reactor biomining have been developed. New applications for microbial and chemical leaching of precious metals from oxide ores, electricity generation by microbial fuels cells and others are attractive applications of these microorganisms.

Several of above-mentioned activities are connected with the solution of essential environmental problems: prevention of the generation and/or treatment of toxic waters polluted by heavy metals, radionuclides, arsenic etc. by means of active and /or passive systems, reactive zones, bioremediation of post-mining landscapes and wastes.

All above-mentioned possibilities for practical applications of acidophilic chemolitotrophic bacteria and archaea, methods for isolation and molecular identification as well as structure of microbial consortium in natural habitats and mineral processing will be also under discussion.

Keywords: chemolithothrophs, acidophilic microorganisms, biomining, microbial pretreatment, mineral biotechnology

EnvM2

CLEAN-UP OF PHENOL BY ENVIRONMENTAL BACTERIA BIOFILMS ENTRAPPED IN PEO CRYOGELS

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Introduction. Two xenobiotic-degrading environmental bacterial isolates - KCM R_5 and KCM RG_5 were cultivated from heavy metal and aromatics polluted industrial area in South Bulgaria. Both strains were studied as free-living cells for their capability to grow on phenol as well as on five chloro- and nitrophenols as a sole carbon and energy source. Further the strains were introduced inside polyethylene-oxide (PEO) cryogels where they easily have biofilmed. An appraisal for their phenol biodegradation was carried out.

Materials and methods. PCR, ARDRA, sequencing and BLAST analysis were applied for the strains' molecular-genetic characterization. Resistance of the environmental isolates to heavy metals (Mn, Pb and Cd), to Cu and Zn and the degradation of phenol derivatives as free-living cells was examined in mineral media, lasting one week. Based on the preliminary results, high molecular PEO (poly-ethylene oxide) cryogels were synthesized according an original method and both bacterial strains were entrapped separately into the cryogels. The last was conducted *via* joint shaking of both components for 48 h at room T^o, resulting in two biofilters formation - PEO-KCM R_5(*Pseudomonas*) and PEO-KCM RG_5(*Bacillus*). Afterwards, a sequencing batch phenol degradation was conducted for 4 weeks at 28 ^oC. Feeding of the biofilters held at every 24 h with 250 mL of uprising phenol concentration solutions - between 300 and 1000 mg/L. Ending the phenol biodegradation experiment, the biofilters's structure was studied using SEM.

Results. The 16S rDNA PCR, ARDRA, sequencing, BLAST analysis and the phylogenetic tree view of the environmental bacterial isolates has shown they decisively affiliated to *Pseudomonas rhodesiae* (KCM R_5) and *Bacillus* subtilis (KCM RG_5), identity between 99.5 and 99.9 %. Both strains showed at different extent a xenobiotic tolerance – to metals, heavy metals and mono-aromatics, including nitro- and chloroaromatics. *P. rhodesiae* KCM R_5 *and B. subtilis* KCM RG_5 were capable to grow on phenol, chloro- and nitrophenols as free living cells for a term of 1 week. Remarkably, the biofilter PEO-KCM R_5 degraded completely phenol at concentration of 1000 mg L^{-1} in contrast to the biofilter PEO-KCM RG_5 which degraded easily 600 mg L^{-1} phenol but did not stand the concentration of 1000 mg L^{-1} and collapsed in an instant time - within 24 h after first portion, already at the the 2^{nd} week of treatment. Strong phenol toxicity harmed its cell structures and processes and killed the whole population. The subsequent SEM analysis revealed that after 2 weeks of continuous harsh phenol treatment of PEO-KCM RG5(*Bacillus)* biofilter and for 4 weeks – of the PEO-KCM R5(*Pseudomonas*) biofilter, the cryogels remained compact, porous, unpierced and elastic and the bacteria entrapped in - swelled and vivid, in vast biofilms.

Conclusions. PEO cryogel-environmental bacteria biofilters are highly effective and suitable for phenol and its chloro-and nitroderivatives' degradation, prospective for clean-up biotechnologies of industrially polluted waters.

Keywords: environmental bacteria, xenobiotics resistance, 16S rDNA sequencing, PEO cryogel entrapment, phenol degradation

Acknowledgements: This work was supported by Grant VUH-302 of The National Science Fund, Bulgarian Ministry of Education and Science

EnvM3

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

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The anaerobic digestion (AD) of organic wastes is a biotechnology which mineralizes different in origin organic wastes and as a result biogas and digestate are produced. AD main advantages are: 1. It solves environmental problems; 2. It solves energy problems; 3. The digestate is natural manure replacing the high energy consuming fertilizers and it can be used in ecological agriculture.

The paper has the goal to present some results of the long-year scientific work of a multidisciplinary team of the Stephan Angeloff Institute of Microbiology over the process of AD of organic wastes (single and mixtures) as well as developing new models and algorithms in order to optimize and control it. The second goal of the paper is to present the current state of our research and some new perspectives. One of them is the so called two-stage AD with simultaneous hydrogen and methane production. It is known that in this two-*stage (*H_2+CH_4) system the energy yields are 20-40% higher compared to the traditional one-stage CH_4 production process. Our recent studies concern two-*stage AD of ligno-cellulosic wastes with and without pretreatment. Some new results were obtained. New mathematical models* of a continuous process of AD with production of hydrogen and methane in a cascade of two bioreactors were developed and investigated. These models are quite simple but presents one possible solution of the very important problem – determination of the optimal ratio (criterion - maximal energy production) of the working volumes of both bioreactors. On the basis of the accumulated knowledge with experimental and simulation research and the models developped we studied new highly efficient algorithms for the AD control.

EnvM4

MICROBIAL BIODEGRADATION AS A TECHNOLOGICAL APPROACH FOR UTILIZATION OF CELLULOSE CONTAINING WASTES DURING LONG-TERM MANNED SPACE MISSIONS

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Aim. To develop a laboratory technology for microbial degradation of organic hygiene cellulosic materials used by the crew of the spacecraft during long-term flights, such as those of the International Space Station and probable flights to Mars.

Methods. Genetic identification of isolated strains using 16S rDNA; anaerobic cultivation techniques applied in bioreactors and jars; determination of cellulose concentration; volatile fatty acids measurement by a gas chromatograph; mathematical modeling.

Results. Microbial community with cellulose degrading potential at mesophilic and thermophilic conditions and different substrates was selected and identified. Biodegradation of cellulose containing components similar in composition to waste hygiene materials used by space crews was estimated. The degree of cellulose utilization was determined as 74,3% at 37 ºC. The dynamics in the degradation of a model substrate (filter paper) by a selected mesophilic microbial community showed most intense degradation during the first 14 days of the process, then by the 22^{-nd} day cellulose degradation rate decreased twice. Concentrations of volatile fatty acids - a major metabolic product in anaerobic biodegradation were investigated. A mathematical model of anaerobic biodegradation with production of hydrogen from organic wastes was also created, including the hydrolysis phase.

Conclusions. Effective degradation of cellulose by selected microbial community at mesophilic conditions was achieved. Mainly accumulated were acetate, propionate and butyrate, which could serve as substrates for the next step in a closed micro-environmental life-support system. Mathematical model for anaerobic biodegradation of lignocellulosic wastes was created.

Keywords: biodegradation**,** cellulosic wastes, manned space flights, life support systems

EnvM5

Interplay between crayfish host, its epibionts and oomycet pathogen *Aphanomyces astaci*, causative agent of crayfish plague

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Aim. Climate change affects freshwater organisms and their interactions, i.e. the pathogens are mostly predicted to increase their distribution. We have studied the complex relationship between crayfish host, its epibiontic communities and its pathogen *Aphanomyces astaci* at different water temperatures. Elevated water temperature caused by climate change simultaneously affects crayfish fitness, composition of cuticle-associated communities and the virulence of its pathogens.

Methods. We have infected two crayfish species, *Austropotamobius torrentium* and *Astacus leptodactylus*, with *A. astaci* zoospores at increased water temperature (22 ^{0}C, simulation of climate change) and control temperature (18 ^{0}) and monitored crayfish mortality. We took cuticle swabs before the experiment and immediately after death to test for *A. astaci* presence. Thus, we could determine the cause of death of each animal (*A. astaci*-induced or other causes). Also, we analyzed the epibiontic microbiome composition by Illumina Miseq.

Results. The composition of cuticle-associated microbial communities was mostly determined by temperature. Further, at increased temperature crayfish mortality was reduced and mostly could not be ascribed to *A. astaci* infection. The observed higher diversity of epibiontic communities at elevated temperature could have contributed to host resistance to *A. astaci*.

Conclusions. We present the first data on cuticle-associated microbiota of *A. torrentium* and *A*. *leptodactylus*, as well as their correlation with temperature and *A. astaci*. This can help to predict the spreading of crayfish plague, as one of the most important causes of decline of indigenous European crayfish, in the future climate change scenarios.

Keywords: climate change, crayfish plague, epibiontic microbial communities, freshwater crayfish

EnvM6

EVALUATION OF THE RISK OF DAMAGE ON DRUPACEOUS FRUIT SPECIES AS THE RESULT OF INFCTION WITH PRUNUS NECROTIC RINGSPOT VIRUS (PNRV, PNRSV) AND PRUNE DWARF VIRUS (PDV)

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Aim. The evaluation aims at the possible damages caused by Prunus necrotic ringspot virus (PNRV, PNRSV) and prune dwarf virus (PDV) (family Bromoviridae, genus Ilarvirus -Isometric Labile Ringspot). Their presence in the infectious background is a threat to economically important drupaceous fruit species (plum, peach, cherry etc.), grown in Bulgaria.

Methods. The article presents an analysis on four levels: epidemiology, pathogenicity, reaction of cultural plant and agrotechnology) considering plant health deterioration, yield reduction and deterioration of fruit quality. The risk of plant health deterioration was examined in the process of the development of the cultural plant.

Results. Examples are given on different reactions to both pathogens according to the cultivar appurtenance of the fruit species. The risk points in the fruit growing are specified with a view to minimizing of the losses as a consequence of infection with PNTV and PDV.

Keywords: PNRV, PDV, infection, damage

EnvM7

BIOLOGICAL ACTIVITY OF EXTRACTS ISOLATED FROM *GEUM URBANUM* L.

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Geum urbanum L. has been used in Bulgarian folk medicine for treatment of inflammatory and infectious diseases of the human gastro-intestinal tract.

Aim. We investigated the antimicrobial, cytotoxic and antioxidant activity of eight extracts on a panel of pathogenic bacterial strains and human cell lines.

Methods. Roots and aerial parts were used to obtain methanol, petroleum ether, ethyl acetate (EtOAc) and *n*-butanol extracts. We calculated minimal inhibitory and bactericidal concentrations (MIC/MBC) by using serial dilution method and dehydrogenase activity by MTT-test. The radical-scavenging potential was investigated by DPPH and SOD methods. Bacterial growth rate was determined by time-kill assay. Anti-QS potential was investigated on *P. aeruginosa* (PA01). The cytotoxicity was tested on the cell lines HEK-293 (human normal transformed kidney embryonal cells) and HEP-G2 (human hepatocytes) by MTT and CFU assays. Antioxidant activity was measured by the total glutathione levels with Ellman's reagent. IC_{50} values were calculated with GraphPad Prism.

Results. All fractions exhibited antibacterial activity against *Staphylococcus aureus, S. epidermidis* and *Bacillus cereus.* The EtOAc extracts showed highest antimicrobial and radical-scavenging activity due to the high percentage of polyphenolic compounds. The IC_{50} on cell lines corresponded to MIC and MBC depending on the extract type. Both, EtOAc and *n*-butanol extracts inhibited the QS system of PA01.

Conclusions. The EtOAc extracts showed the highest antimicrobial, cytotoxic and antioxidant activities compared to the other extracts. *G. urbanum* is a perspective new source of bioactive compounds and deserves further, more detailed studies.

Keywords: *Geum urbanum,* plant extracts, antibacterial activity, quorum sensing, cytotoxicity

Acknowledgements: We thank for the support of this study by DFNP-70/27.04.2016 of the Bulgarian Academy of Sciences for young researchers given to Assist. Prof. Lyudmila Dimitrova and **by** the Alexander von Humboldt Foundation Grant "Equipment subsidies" to Assist. Prof. Maya M. Zaharieva.

EnvM8

MICROBIOTA IN ATMOSPHERIC AEROSOLS DURING LIDAR MONITORING OF DENSELY POPULATED URBAN AREA IN SOFIA

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Aim. The aim of the study is to analyzed of microbiota during the investigation on particulate matter (PM) in atmospheric aerosol over a densely populated urban area of Sofia.Combination between lidar monitoring over a densely populated urban area of Sofia, with on-the-spot sampling at sites with high concentration of dust products was used. The latter was performed by the three-wavelength lidar system at operational distances exceeding 25 km

The collected dust material was subjected to microbiological investigation. Pure cultures of bacterial and fungal isolates were identified on the basis of morphological, physiological and biochemical characteristics as well as molecular approach, including the amplification of 16S rDNA, resp. 18S rDNA for fungal isolates and sequencing.

The microbiological survey of the atmosphere indicated that the collected particulate matter harbors a variety of different viable microorganisms. The majority of the bacterial isolates is presented by predominantly saprophytic pigmented bacteria, mainly from the genera *Micrococcus* (66%). The genus *Bacillus* was also dominated, presented by representatives of the species *B. pumilus*, *B. cereus*, *B. thuringensis* and *B. cereus*. One isolate was determined as *Staphylococcus equorum*. The fungal presence was presented mainly by the genera *Penicillium, Aspergillus*, *Cladosporium* and *Symmetrospora*.

The approach used is effective for analyzed the micron-sized particles when the PM is dispersed as aerosols and created conditions favoring the occurrence of chemical and bioprocesses. **Keywords**: LIDAR monitoring, micron-sized particles, air born microorganisms, air microbiology

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EnvM9

ISOLATION OF THE BACTERIA FROM GENUS *GEOBACTER* AND APPLICATION IN MICROBIAL FUEL CELL FOR ELECTRICITY GENERATION

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Aim. The aim of the study is to isolated bacteria from genus *Geobacter* from natural habitats, investigated the different organic substrates for oxidation as well the suitable electron acceptors and use the isolates in microbial fuel All isolates were subjected to molecular identification performed by isolation of DNA, the amplification of 16S rDNA The fuel cell used in this study was a Plexiglas cylindrical column 80 cm high, a perforated slab graphite-Mn^{4+} anode and a graphite-Fe^{3+} cathode located in the bottom and in the top section of the column, respectively. The two sections were separated by a permeable barrier.

Geobacter metallireducens, Geobacter pelophilus and *Geobacter sulfurreducens.*

To optimize the growth of the isolates different carbon sources were tested. It was found that the best substrate for oxidation is acetate.

As a terminal acceptor of the electrons in respiratory chain these bacteria are able to use ferric ions or fumarate (iron and fumarate respiration respectively). It was found that all isolates tested performed mainly iron respiration.

For use in microbial fuel cell *Geobacter sulfurreducens G1* was tested efficient electricity generation in the microbial fuel cell – up to 1000 mW/m^2 was registered.

Geobacter sulfurreducens G1 is suitable for efficient electricity generation.

Keywords: *Geobacter, Geobacter sulfurreducens G1*, microbial fuel cell; Electrochemically active microorganisms, classical taxonomy, molecular taxonomy

Acknowledgments: The Bulgarian National Science Fund under project T02/2 supported this study.

EnvM10

OCCURENCE AND ANTIMICROBIAL RESISTANCE OF *ENTEROCOCCUS* SPP. FROM BULGARIAN GROUNDWATER SOURCES

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Aim. The aim of the study was to isolate, identify and determine the antibiotic susceptibility profiles of *Enterococcus* spp in groundwater sources from Stara Zagora region, Bulgaria.

Methods. The membrane filter method was used to determine enterococci in 100 ml sample. A sterile forceps was used to remove the membrane filters from the machine. These filter papers were placed on ready to use, selective and chromogenic plate (Compact Dry ETC). After 24 h incubation period at 37^0 C, randomly selected typical blue to blue-green colony were sub-cultured on *Enterococcoccus* Agar. The identities of the isolates were determined using EN-COCCUS test kit (MIKROLATEST® ID). Disc diffusion method was used to detect resistance to ampicillin, tetracycline, streptomycin, erythromycin and gentamicin.

Results. Among 23 tested isolates, 5 enterococcus species were identified; *E. faecalis* (34.8%), *E. pseudoavium* (34.8%), *E. durans* (17.3%), *E. cecorum* (8.6%) and *E. avium* (4.3%). A large proportion of the strains are resistant to streptomycin (39%); 43.4% are intermediate to resistant against erythromycin. We found that two strains of *E. pseudoavium* are multidrug resistant (tetracycline, streptomycin, and erythromycin). Only one strain of *E. durans* was resistant to ampicillin. There are no resistances to gentamicin among tested strains.

Conclusions. From the obtained results, it can be concluded that, the level of contamination of ground water with *Enterococcus* species and their species composition are very high. The detection of large numbers of resistant isolates was of concern as this could lead to the dissemination of antimicrobial resistant strains that have negative clinical implications.

Keywords: *Enterococcus* spp., antimicrobial resistance, ground water

EnvM11

MICROBIAL PRETREATMENT OF A GOLD-BEARING SULPHIDE

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Aim. The aim of this investigation is to subjected a gold-bearing sulphide concentrate to microbial pretreatment to liberate the precious metals from sulphide matrix of the concentrate and to expose them to subsequent extraction by means of suitable reagents (tiosulphate, thiourea, cyanide)

Methods. Samples of a gold-bearing pyrite-arsenopyrite concentrate containing 32g/t gold and 820 g/t
silver as most valuable components were subjected to microbial pretreatment to liberate the precious metals from sulphide matrix.

The concentrate pretreatment was performed initially by the shake-flask technique and then in agitated bioreactors by means of selected extreme thermophilic archaea of the genera *Metallospaera, Sulfolobus* and *Thermoplasma* at 86 ^{0}C and pulp density of 5 - 20%.

Results. The oxidative activity of the microbial cultures used in this study, a part of the temperature was affected by some environmental, i.e. technological, factors as pH, degree of aeration, presence and concentration of nutrients, pulp density, temperature and stirring rate.

It was found that sulphide oxidation of 45 – 95 % was sufficient for achieving extraction of about 92–98 % for gold and 88–95 % for silver.

It must be noted that only 9–12 % from the gold and 5–7 % from the silver were extracted from initial non-pretreated by microbial oxidation sulphide concentrate.

Conclusions. The efficient microbial pretreatment of sulphide concentrate containing precious metals (gold and silver) finely enclose in the sulphide matrix is an essential stage of processing of such concentrates

Keywords: gold, sulphide, chemolithothrophs, microbial pretreatment

EnvM12

BIOLEACHING OF CHALCOPYRITE CONCENTRATE BY MEANS OF CHEMOLITHOTROPHIC MICROORGANISMS FROM DIFFERENT TAXONOMIC GROUPS

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Aim. The aim of this study was to compare the ability of acidophilic chemolithothrophic microorganisms (bacteria or archaea) with different temperature requirements for an efficient growth and oxidative activity during the bioleaching of chalcopyrite concentrate.

Methods. The concentrate used contained 31.8% Cu, 30.2% Fe and 35.0% S as the main components.

The mesophilic cultures consisted of *Acidithiobacillus ferrooxidans*, *A. thiooxidans* and *Leptosprillum ferrooxidans,* the moderate thermophilic cultures of *Sulphobacillus thermosulphidooxidans* and *Acidithiobacillus caldus* and extreme thermophilic cultures consisted of archaea from genera *Methallosphaera, Sulpholobus* and *Thermoplasm*a were used.

The bioleaching experiments with the concentrate were performed by shake-flask technique and in agitated bioreactor. The pulp density varied from 5 - 30%.

Results. Microbial cultures tested differed considerably from each other with respect to their ability to leach chalcopyrite. However, it must be noted that each temperature group contained microbial cultures able to extract more than 95% from the copper. The rates of copper extraction varied to a great extent even within the cultures tested at one and same temperature.

The most efficient for the leaching of chalcopyrite with a low pulp density (5-10%) were the archaea. The moderate thermophilic bacteria were the most efficient in systems with high pulp density (15 - 30%).

Conclusions. The acidophilic chemolithotrophic microorganisms differ considerably with respect to their ability to leach chalcopyrite and others minerals. The preliminary adaptation of the microorganisms to relevant mineral substrates is essential for approach for performing the efficient leaching.

Keywords: chalcopyrite, bioleaching, acidophilic chemolithotrophic microorganisms

EnvM13

MICROBIAL CLEANING OF POLLUTED WATERS CONNECTED WITH ELECTRICITY GENERATION

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Aim. The main aim of this investigation was to combined the treatment of polluted waters by means of a passive system with subsequent treatment of the effluents from this system enriched in soluble biodegradable organic compounds by means of a microbial fuel cell to generate electricity

Methods. The passive system consisted of an alkaline limestone drain and permeable reactive multibarrier connected in a series. The multibarriers was filled by mixture of solid biodegradable organic substrate (mainly of plant biomass) and was inhabited by different heterotrophs including various sulphate-reducing and iron reducing bacteria. The effluents from the miltibarrier were treated in a microbial fuel cell.

Results. The water treatment by means of a passive system was very efficient during the different climatic seasons. The microbial dissimilatory sulphate reduction was the main mechanisms involved in the water clean-up. However, some bacteria possessing anaerobic iron respiration reduced the ferric iron to the bivalent state and in this way prevent the precipitation of this element at pH about 6 – 7.

The treatment of the effluents from the multibarrier resulted in an efficient electricity generation in the microbial fuel cell – up to 1200 mW/m^2.

Conclusions. The combination of the water treatment by means of a passive system with electricity generation by means of a microbial fuel cell is an efficient way to make the water cleaning and electricity generation very efficient.

Keywords: cleaning of polluted waters, electricity generation, microbial fuel cell

Acknowledgments: The Bulgarian National Science Fund under project T02/2 supported this study.

EnvM14

MUCUS FROM *CORNU ASPERSUM* – A PERSPECTIVE RESOURCE OF ANTIOXIDANT SUBSTANCES

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Introduction. Snail mucus is a rich source of bioactive natural compounds with a variety of functions, including facilitating of the treatment of wounds and skin conditions. More importantly, the mucus is well known for its anti-aging properties and frequently investigated for antibacterial potency. Generally, both of these properties facilitate the ability of the organism to cope with the generation of free radicals.

Aim. The aim of the present study was to evaluate the extent of antioxidant potential of fractions from mucus of garden snail *Cornu aspersum.*

Methods. The model snail species were collected in Bulgaria and the mucus was purified and subjected to ultrafiltration. Three different samples were investigated – lyophilized fractions (molecular mass < 10 kDa and > 20 kDa) and liquid fraction (> 20 kDa). Inhibitory effect of the fractions was determined by calculating of the inhibition of NBT reduction by photochemically generated superoxide. Superoxide dismutase (SOD) activity and dose-dependence of the superoxide anion scavenging effect were studied as well.

Results. The fraction with molecular mass <10 kDa showed the highest SOD activity. It is possible that the detected SOD value represents the so called “SOD-like” activity that could be displayed by some peptides with low molecular mass capable of superoxide scavenging.

Conclusions. The results from the antioxidant screenings of *C. aspersum* mucus and its fractions show that this naturally derived product (specifically the low molecular weight fractions), has a good potential to counteract the formation of reactive oxygen radicals.

Keywords: *Cornu aspersum*, superoxide radicals, SOD, antioxidant activity

Acknowledgment: This research was carried out with the support of a project under contract No. DN 01/14 of 19.12.16, funded by the Scientific Research Fund of the Ministry of Education and Science in the Republic of Bulgaria.

EnvM15

New plant host of *Pseudomonas viridiflava* in Bulgaria

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Aim. It has long been assumed that *Leucojum aestivum* (summer snowflake) does not suffer diseases due to the antimicrobial properties of its extracts. However, during an annual observation, leaf disorders of a natural population were observed. The aim of this study was to identify the causal agent of the disease of *L. aestivum*.

Methods. Isolations were carried-out according to the standard procedure. The pathogenic potential of the bacteria was examined by the hypersensitive reaction test and artificial inoculations of *L. aestivum* and *A. sativum*. The symptoms of disease were observed periodically between the 1st-7th day. Microlog™ 4.20.05 (Biolog™), PCR with species-specific primers and sequencing of the gene for 16S rRNA were used for identification of the pathogenic strains.

Results. Overall twelve bacterial strains were isolated and subjected to subsequent analyses. Damages on *L. aestivum* and *A. sativum* scales were clearly seen on the 5th day after inoculation. The pathogenic strains were identified by Microlog™ as *Pseudomonas viridiflava* by probability 100%. All of these strains showed the specific for the species amplification product of 180bp. The sequences of the strains showed highest similarity with *P. viridiflava* strains.

Conclusions. *L. aestivum* is reported as a plant host of *P. viridiflava* for the first time.

Key words: *Leucojum aestivum,* summer snowflake, *Pseudomonas viridiflava,* disease

EnvM16

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR-GENETIC CHARACTERIZATION OF BACTERIAL STRAINS ISOLATED FROM SPRING AND HEALING WATERS IN THE REGION OF HASKOVO

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Aim. The aim of the present study was to detect of physiological, biochemical and molecular-genetic characteristics of new isolated bacterial strains from spring and healing waters in region of Haskovo.

Methods. The biochemical profiles of the selected bacteria are determined with the system for rapid identification API 50 CH. The strains are identified by software processing of the obtained biochemical profiles with Apiweb®. Furthermore, the tested strains are genetically identified by molecular-genetic methods – ARDRA analysis and sequencing of the gene for the 16S rRNA.

Results. 13 new rod shaped, Gram–positive and spore forming bacteria was isolated from spring and healing waters in the region of Haskovo. The colonial and physiological characteristics of the isolated strains are defined.

Conclusion. The results obtained from the biochemical and molecular-genetic studies indicate that the selected strains belong to different *Bacillus* species.

Keywords: microflora, healing waters, mineral waters, selection, identification, sequencing, *Bacillus* spp.

EnvM17

PHAGE SENSITIVITY OF BULGARIAN PHYTOPATHOGENIC XANTHOMONADS ISOLATED FROM TOMATO AND PEPPER PLANTS

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Aim. The present study aims to identify the sensitivity of Bulgarian phytopathogenic xanthomonads to a phage biopesticide recommended for biocontrol of bacterial spot disease on tomato and pepper.

Methods. Fifteen bacterial strains were used in this study: *Xanthomonas vesicatoria* (6), *Xanthomonas euvesicatoria* (5) and *Xanthomonas gardneri* (4). All strains were isolated from infected tomato and pepper plants and were previously identified. The selection was made based on their pathotype and races in all available combinations. Their sensitivity was tested by spot – inoculation of the phage preparation onto medium surface, previously inoculated with the target phytopathogenic bacteria. The positive results were scored after forming of clear plaques from bacteriophages as a result of the lysis of sensitive bacteria.

Results. This study established low sensitivity of *X. vesicatoria* strains as only one strain turned out to be sensitive biopesticide to the phages included in the biopesticide. This strain was tomato pathotype and race T3. The same result was obtained for *X. gardneri* strains as the sensitive isolate was pepper – tomato pathotype and race T1. In contrast, all *X. euvesicatoria* strains showed sensitivity to the studied biopreparation no matter of their pathotype and race.

Conclusions. The tested could be effective against *X. euvesicatoria,* but not against the rest two bacterial spot causing xanthomonads (BSCX). However, in Bulgaria this disease is caused by the three aforementioned species and for effective phage - biocontrol a preparation containing phages active against all BSCX is needed.

Keywords: bacteriophages, bacterial spot, *Xanthomonas vesicatoria*, *Xanthomonas euvesicatoria*, *Xanthomonas gardneri*

EnvM18

A THERMOPHILIC BACTERIUM FOUND IN ANTARCTICA

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Aim. Antarctica is the coldest and the least researched continent on the Earth. In the last decades the microflora of the coldest regions in the world has been intensively studied. The aim of the present study is species identification of two newly isolated strains from penguin excrements on Livingston Island, Antarctica.

Methods. The strains were initially identified by A. Gousterova as genus *Thermoactinomyces* and described as strains *Thermoactinomyces* sp. IMBAS-14A and *Thermoactinomyces* sp. IMBAS-22A. A total DNA from the both strains is isolated by a protocol of QIAGEN. A molecular characterization of the both strains by16S rDNA-PCR amplification, followed by sequencing and BLAST analysis was done.

Results. As a result of a BLAST comparative analysis it was found 99% similarity of the both strains *Thermoactinomyces* sp. IMBAS-14A and IMBAS-22A to the species *Thermoactinomyces sacchari* which is with new taxonomic name *Laceyella sacchari*. The same high percentage of identity was found to the species *Laceyella tengchongensis* too. The molecular approach was combined with other methods to distinct both species. *Laceyella tengchongensis* doesn't break down starch and has maximal growth temperature 70° C, but *L. sacchari* does it and has maximal growth temperature of 65° C. It is established experimentally that the strains *Thermoactinomyces* sp. IMBAS-14A and IMBAS-22A break down starch and have maximal growth at 60° C. Therefore, the both strains belong to the species *Laceyella sacchari*.

Conclusion. This data expands the scientific information about the new habitats of this species in one of the coldest regions of the world.

Keywords: thermophilic bacterium, Antarctica, penguin excrements

Virology

Vir1

HEPATITIS E VIRUS INFECTION: TURKEY VS EUROPE

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Hepatitis E virus (HEV) infection is an important cause of viral hepatitis worldwide. HEV is largely a zoonotic infection and not limited to certain developing countries. The surveillance report of HEV infection published by the European Centre for Disease Prevention and Control (ECDC) in 2017 shows 10- fold increase in reported HEV cases over the last 10 years in Europe. Although the surveillance differs between countries, the majority of the cases are reported from France, Germany and the UK. In recent years, it has become apparent that there are 'hotspots' of HEV infection such as southwest France, central Italy, central/western Poland. Food-borne transmission of the virus appears to be a major route in Europe since most HEV infections are related to the consumption of undercooked or raw pork meat and liver.

There are four major HEV genotypes. Type 1 and 2 are restricted to human beings while type 3 and 4 may infect humans and other mammalian species such as pigs and deer. Besides acute infection, HEV genotype 3 may cause chronic hepatitis and cirrhosis in immunosupressed patients especially solid organ transplant recipients, HIV positive people, patients receiving chemotherapy due to hematologic malignancies. HEV infection after the organ transplantation may cause chronic infection in up to 60% of the patients if not treated. After recognition of chronic HEV infection, increasing number of studies from Europe and USA are published regarding HEV infection in solid organ transplant recipients in the recent years.

Turkey is like a bridge between HEV endemic and non-endemic regions with a HEV IgG prevalence of 3 to 34% depending on geographical location, socioeconomic level, age and other risk factors. A recent multi-center study of solid organ transplant recipients in Turkey done by our group showed that the prevalence of HEV-IgM was 0.8% and HEV-IgG was 15.4% associated with male gender, liver tx and older age. No evidence of chronic infection was detected since none of the patients were HEV RNA positive.

More research on the epidemiology and control of HEV is needed to understand the burden of this emerging, under-recognised pathogen.

Vir2

ANTIENTEROVIRAL ACTIVITY OF NEWLY SYNTHESIZED DIARYL ETHERS

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Aim.Screening for anti-enteroviral activity of 71 newly synthetized derivatives of the molecule 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile (MDL-860) against poliovirus 1, Coxsackievirus B1, and Coxsackievirus B3 strains, replicated in monolayer HEp-2 cell cultures.

Methods. Cells and viruses- Cells and viruses were from the cell culture collection of the Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria.

Cellualar toxicity - the neutral red uptake assay based on the initial protocol described by Borenfreund and Puerner (1984) was used.

Antiviral activity - antiviral screening was based on the viral yield reduction technique.

Coxsackievirus B1(CVB1) infection in newborn mice **–** groups of newborn mice (30 mice per group) were inoculated subcutaneously with CVB1, 20 LD_{50}.

Results. Three of the tested derivatives of MDL-860, 37e, 44e and 45e showed a marked antiviral potential – against PV-1 and CVB1. The compounds 27e and 35e manifested an effect against CVB3. The selected in the in vitro screening compounds 27e, 29e, 35e, 37e, 44e and 45e were tested in experimental CVB1 neuroinfection in newborn mice, infected with 20 LD_{50} virus inoculum. Compound 37e demonstrated the highest activity.

Conclusion. The synthesis and anti-enteroviral activity screening of 71 new analogues of MDL-860 was described. It was found that a marked efficiency is usually demonstrated by two- or three halogen-substituted diarylethers. The future synthesis of new active diarylethers could be promising and has to be directed to other halogen-substituted analogues of MDL-860.

Keywords: enteroviruses, MDl-860, diaryl ethers

Vir3

PATHOGENIC COMPLEXES IN PLANTS AND THEIR SILENT PLAYERS

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Aim. Traditional views on plant diseases are mainly focused on a single etiological agent. Our case studies exemplify pathogenic complexes determining the host health status; 1. oilseed rape with phyllody disease harbouring '*Candidatus* Phytoplasma asteris' and *Turnip mosaic virus* (TuMV) and 2. *Plectranthus scutellarioides* (coleus) with fasciation hosting '*Ca*. P. solani', *Coleus blumei viroid 1* (CbVd-1), CbVd-3 and *Coleus vein necrosis virus* (CVNV).

Methods. Phytoplasmas were characterized from total nucleic acids by amplification and analyses of 16S rRNA gene and multigene sequence analyses of 4-5 other genes. Viroids and viruses were identified from extracted RNAs by RT-PCR and phylogenetic analyses. Virus presence was also tested biologically. In coleus, high throughput sequencing (HTS) was performed on Illumina Nextseq 500 platform.

Results. In the rape displaying phyllody disease symptoms, '*Ca*. P. asteris' was confirmed and the phytoplasma had significant SNPs in the *amp* gene encoding a specific protein essential for aphid transmission. TuMV co-infecting rapes, from the World-B phylogenetic lineage, added to the disease severity. In coleus with fasciation, a pathogenic complex consisted of two viroids (CbVd-1 and -3), a phytoplasma ('*Ca.* P. solani'), and a carlavirus (CVNV). The virus presence was first deduced from HTS data and later confirmed by RT-PCR and biotests. Even though CVNV was expected to cause some symptoms in coleus, it remained silent in this pathogenic complex.

Conclusions. Plants may harbour diverse pathogenic complexes causing diseases or asymptomatic infections. HTS facilitates the discovery of these complexes prompting us to revisit the paradigms of plant-pathogen interactions.

Keywords: coleus fasciation, oilseed rape phyllody, phytoplasmas, viruses, viroids

Vir4

GENE SILENCING AND ENTEROVIRUSES

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Aim. Gene silencing is a phenomenon that regulates gene expression of a certain gene. There are three types of gene silencing – meiotic, transcriptional and post-transcriptional.

The aim of this study is to silence selected gene regions of Enteroviruses by posttranscriptional gene silencing and thus blocking the viral replication.

Methods. In our experiments, we used polymerase complex of bacteriophage Phi6 to produce specific conserved dsRNAs from 5’UTR, 2A and 3D, complimentary to preselected conserved gene regions from the Enteroviral genome.

dsRNA was synthesized by the combination of in vitro transcription and replication of DNA template. We used the enzyme Power Cut Dicer, which cut dsRNA, yielding fragments with a length of 25-27 nucleotides, which resulted in accumulation of a pool of siRNAs.

Results. All the concentrations of the three used fragments HEp-2 cell monolayer. From 0.01 to 0.35 μM absolutely no cytotoxicity was observed for the used dsRNAs and siRNAs.

0.3 μM of all tested dsRNAs and siRNA pools completely supress CVB3 replication in vitro.

Conclusions. The used concentrations of dsRNAs and siRNAs block the viral replication of Enteroviruses in vitro to almost 100 % with a Selectivity Index ranging from 430 to 770.

Keywords: gene silencing, enteroviruses, siRNA

Acknowledgements: This study was supported by National Science Fund, Bulgaria by contract NoDN 11/12 from 18.12.2017.

Vir5

DOUBLE ANTIVIRAL COMBINATIONS APPLIED BY CONSECUTIVE ALTERNATING ADMINISTRATION AGAINST COXSACKIEVIRUS B1 INFECTION IN MICE

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Aim. Human enteroviruses, distributed worldwide, are causative agents of a broad spectrum of diseases with enormously high morbidity, including a series of severe illnesses that affect the CNS, heart, □-cells of pancreas, skeletal muscles, and so on. Unfortunately, there is no specific treatment or vaccine available for these infections, and the patients' treatment is mainly supportive. In the last few years our team has developed an experimental alternative treatment strategy based on consecutive alternating application (CAA) of inhibitors with different modes of action. This work represents the antiviral activity of double combinations of anti-enteroviral compounds applied via CAA course against the Coxsackievirus B1 neuroinfection in mice.

Methods. Antiviral combination effects were examined by relying on double combinations of pleconaril, guanidine hydrochloride, MDL-860 and oxoglaucine put through CAA treatment scheme on CVB1 neuroinfection 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.

Results. The results of these analyses indicate markedly improved efficacy of PG, PO and PM combinations administered according to the CAA treatment schedule in CVB1 infected mice - decreased mortality rate and lengthening of the mean survival time (MST). MG and MO applied consecutively were ineffective. In comparison with placebo groups the monotherapeutic course with pleconaril demonstrated some independent antiviral effect. It was found that MDL-860, oxoglaucine and guanidine.HCl monotherapies were without a marked antiviral effect.

Keywords: double combinations, *in vivo*, Coxsackievirus B1

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Vir6

ANTIVIRAL ACTIVITY OF DOUBLE DRUG COMBINATIONS WITH ANTI-ENTEROVIRALS VERSUS COXSACKIEVIRUS B1

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Aim. Coxsackieviruses, part of the *enterovirus* genus, can cause severe diseases of the heart, liver, eyes and pancreas, as well as acute infections of the central nervous system. Coxsackievirus B (CVB) infections are common in children and young adults. Until now, there are no enterovirus-specific drugs available for clinical use. The main reason for that is the fast development of drug-resistant and even drug-dependent mutants. Use of synergistic combinations of antivirals might be one of the possible efficient approaches to overcome the disadvantages of monotherapy and may restrict the emergence of resistance to either or both of the partners in the combination. We investigated the effects in cell culture based on combination of inhibitors with different mode of action and fluoxetine (Prozac®).

Methods. Double combinations by fluoxetine with pleconaril, MDL-860, guanidine hydrochloride, HBB, and oxoglaucine were tested for their activity against Coxsackievirus B1 strain Connecticut-5. Antiviral combination effects due to drug–drug interaction were examined by relying on the three-dimensional model developed by Prichard and Shipman using MacSynergy™ II program.

Results. The combinations of fluoxetine with pleconaril, MDL-860 and oxoglaucine exhibited additive effect, while combinations with guanidine hydrochloride and HBB revealed minor synergy at the 95% confidence level. The highest volume of synergy was observed when fluoxetine was combined with HBB. Combinations of pleconaril, MDL-860, HBB, oxoglaucine and guanidine hydrochloride with fluoxetine had no effect on the viability of uninfected HEp-2 cells.

Conclusions. The resistance occurring after monotherapy with a certain anti-enteroviral drugs makes it reasonable to focus interest on combined administration of antivirals.

Keywords: double combinations, fluoxetine, Coxsackievirus B1

Vir7

EFFECT *IN VITRO* OF DOUBLE DRUG COMBINATIONS AGAINST COXSACKIEVIRUS B3

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Aim. Coxsackievirus B3 (CVB) is one of the major causative pathogens of viral myocarditis. Although most CVB-related cardiac illnesses are subclinical, severe viral myocarditis can lead to heart failure or sudden cardiac death. There are no enterovirus-specific drugs available for clinical use because of fast development of drug-resistant and even drug-dependent mutants. Synergistic combinations of two or more agents can overcome toxicity and other side effects associated with high doses of single drugs, by either countering biological compensation, sparing doses on each compound, or accessing context-specific multi-target mechanisms, also may restrict the emergence of resistance to the partners in the combination.

Methods. We studied the combined effects in cell culture of inhibitors with different mode of action against cardiotropic Coxsackievirus B3 (Woodruff strain). Theoretical additive interactions of expected effects for drug–drug interactions was calculated by using MacSynergy II (Prichard et al.,1993). Interpretation of significance of the observed volumes of synergy or antagonism, depicted in the differential surface plots, was based upon the program guidelines.

Results. An additive effect was exhibited when pleconaril and guanidine hydrochloride were combined with oxoglaucine. The combinations of pleconaril with guanidine and MDL-860 manifested moderate synergy, while combinations of MDL-860 with oxoglaucine and guanidine revealed strong synergy. Combinations of pleconaril, MDL-860, oxoglaucine and guanidine hydrochloride had no cytotoxicity effect on uninfected HEp-2 cells.

Conclusions. The increased specificity of combinations over single agents has implications for drug discovery and bioengineering. In medical contexts, the selectivity bias reinforces the potential of chemical combinations for network polypharmacology in enteroviral infections.

Keywords: double combination, pleconaril, Coxsackievirus B3

Vir8

HBV AND HCV INCIDENCE AMONG NON-HODGKIN'S LYMPHOMA PATIENTS IN VARNA REGION (2013-2016)

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Aim. The aim of this study is to find out the frequency of patients with non-Hodgkin's lymphomas and hepatitis B (HBV) and C (HCV) infections in Varna region. The numerous epidemiological studies runned, in recent years, emphasize the relationship between hepatitis B and C and non-Hodgkin's lymphomas. Bulgaria has a higher incidence of hepatitis B (3.87% of HBsAg carriers) when compared with hepatitis C (1.28%). In one national survey conducted in the period 1999-2000, the incidence of these infections in the region was higher than the average rate for the country- 5.26% and 1.4%, respectively.

Materials and methods. We analyzed 466 patients with non-Hodgkin's lymphomas in the laboratory of clinical virology at "St. Marina University Hospital", Varna. Standardized and sensitive ELISA assays were used for testing the serological markers HBsAg, anti-Hbc total Ab and anti-HCV Ab. All of the participants in the survey were tested for HBV, 60 of them for anti-Hb total Ab and 462 for anti-HCVAb.

Results. Positive for anti-HCV Ab were 1.7% (95% CI: 0.8-3.4, n = 8) and 8.2% (95% CI: 5.8-11.0, n = 38) for HBsAg. Fifty of the HbsAg negative patients were tested for anti-HBc total Ab and positive results were found in 46.0% of them (95% CI: 31.8-60.7, n = 23).

Conclusion. As per our research the incidence rate of HBsAg positive patients with non-Hodgkin's lymphoma is higher, when compared with the endemicity of the Varna region and Bulgaria. The results obtained for non-Hodgkin's lymphoma patients positive for anti-HCV Ab did not differ significantly from the information in the literature. This data have shown the greater prevalence of HBV in patients with non-Hodgkin's lymphoma against HCV in our region.

Keywords: Hepatitis B, Hepatitis C, Non-Hodgkin's lymphoma.

Vir9

INVESTIGATION OF ANTI-EA (D) IGG IN PATIENTS WITH HAEMATOLOGICAL DISEASES

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Aim. Epstein-Barr virus is associated with a wide range of clinically relevant diseases - from infectious mononucleosis, to malignant diseases of epithelial and lymphoid origin. The use of serological markers, such as predictors of malignant disease or the detection of viral reactivation, are debatable and unsupported by many studies. The purpose of this study is to establish positivity in the anti-EA (D) IgG test in patients with haematological diseases as a possible marker for viral reactivation.

Materials and Methods. We examined 91 patients with haematological diseases (acute leukemias and non-Hodgkin's lymphomas, including chronic lymphocytic leukemia), of which 54.9% (95% CI: 44.2-65.2) men. The age range is from 1 to 83 years, with predominance among older patients. Patients were tested for anti-EA (D) IgG with tests of Euroimmun, Germany.

Results. We found 13.2% (95% CI: 6.2 -20.1, n = 12) positive, of which 66.7% (95% CI: 40.0 -93.3, n = 8) had chronic lymphocytic leukemia. Two of the patients are in a gray zone. All positive patients are over 60 years of age.

Conclusion. Positive anti-EA (D) IgG tests represent a small relative share. Patients with chronic lymphocytic leukemia predominate.

Keywords: Epstein-Barr virus, anti EA(D)IgG, leukemias, non-Hodgkin's lymphomas.

Infectious Immunology

II1

THE UNIVERSE OF PROTEINS AS SEEN BY IGM ANTIBODIES

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The repertoire of human IgM producing B cells contains at any given moment approximately 10^6 clones. Most of them are naïve B cells producing natural antibodies. Their task is to provide first line of defense while the adaptive response initiated from their pool produces high affinity/ high specificity antibodies. At the same time, this antibody repertoire compartment proves involved also in regulatory and homeostatic functions. Therefore, it can be used as a natural biosensor of the antigenic landscape of the body's internal environment. The rich set of polyspecific, moderately autoreactive variable domains of the natural antibodies prove instrumental to this task. In an attempt to create a tool to study the dynamics of the human IgM repertoire recently we developed a library of 220 000 7-mer peptide mimotopes of common (or also public) specificities found in a pool of IgM from 10 000 healthy donors. Besides its practical utility as a source of igome biomarkers for oncology, autoimmunity etc., the rationally designed mimotope library carries a rich structural information about the epitopes of IgM. Here we shall review the recent results produced as we compared the epitopes of high affinity antibodies and these of natural IgM using bioinformatics and artificial intelligence.

II2

HOMEOSTASIS OF NEUTROPHILS IN INFLAMMATATORY AND DEGENERATIVE JOINT DISEASES

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Aim. The role of neutrophils in the pathogenesis of joint inflammatory and degenerative diseases has been recently revisited. Our aim is to investigate neutrophil homeostasis during the progression of joint destruction and to evaluate the therapeutic potential of histone deacetylase inhibitors or activators on neutrophils in experimental models of joint damage.

Methods. In our study Balb/c or ICR mice were injected at the knee cavity with collagenase (5 U/knee) or zymosan (10 μg/knee). Mice were treated at different schedules intraperitoneally, subcutaneously or intraarticularly with the histone deacetylase inhibitor trichostatin A (TSA) or activator (STAC2183). Joint pathology was assessed by Safranin O staining of paraffin-embedded samples. Neutrophil cellularity and expression of homing/activation markers were studied by flow cytometry. Production of nitric oxide (NO), reactive oxygen species (ROS), myeloperoxidase (MPO) and activation of caspase pathway (PARP cleavage) were determined by colorimetric assays.

Results. We found that TSA inhibited bone remodeling and alleviated the disease. However, TSA-treated mice with osteoarthritis showed increased granulopoiesis and expression of chemokine receptors on the surface of bone marrow neutrophils. While TSA administration decreased NO and ROS production, it enhanced MPO expression and degranulation by osteoarthritic/arthritic neutrophils. By contrast the STAC2183 failed to significantly impact PARP cleavage, NO production and neutrophil cellularity in the bone marrow.

Conclusion. Our results suggest that TSA might be an efficient agent for OA therapy hence its action on neutrophils indicates more complex and multifaceted effects barely observed after the application of the STAC2183.

Keywords: neutrophils, inflammation, homeostasis, joint pathology

Acknowledgment: The work has been supported by DN 5/11-2017 grant of National Science Fund.

II3

IGOME ANALYSIS OF THE IGM REPERTOIRE IN GLIOBLASTOMA

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The search for novel biomarkers in oncology is still very intense, answering the demand both for diagnostic approaches as well as a part of the understanding tumor biology and immune surveillance. In response to this demand, we intend to study the structure and dynamics of the IgM repertoire in patients with glioblastoma.

We wanted to find sets of peptide mimotopes, the reactivities to which vary in a similar manner across patients creating diagnostic profiles that can be used as biomarkers of forms, stages and treatment response of the disease.

Here we describe a deep panning approach for the rational selection of a set of peptide mimotopes that represents optimally the diversity of human IgM reactivities using a relatively small library of probes. A 7-mer random peptide phage display library was panned on pooled human IgM. A non-exhaustive set of mimotopes was defined using next generation sequencing of the selected phage. A set of mimotopes representative of the most significant clusters was used to test the hypothesis that this approach samples uniformly the IgM repertoire. In this respect, the representative library is considered an optimal probe of the human IgM diversity. Thus, an optimized library of IgM mimotopes is found to address very efficiently the dynamic diversity of the human IgM repertoire, providing informationally dense, structurally interpretable, profiles of the repertoire's reactivities.

Food Microbiology

FM1

CLIMATE CHANGES AND FOODBORNE PATHOGENS

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Foodborne zoonoses are a significant and widespread public health threat – annually more than 315,000 human cases are confirmed and reported in the EU, but the real number is likely to be much higher. The most frequent bacterial pathogens in the food chain belong to the genera *Campylobacter* (229,213 human cases for 2015), *Salmonella* (94,625 human cases in 2015), *Yersinia* (7,202 human cases or 2015) and the species *Escherichia coli* (5,910 human cases in 2015) and *Listeria monocytogenes* (2,206 human cases in 2015). Many of those survive and proliferate both in the environment and in foods of plant and animal origin, regardless of storage conditions (cold temperature, vacuum packs, salt concentrations, etc.). Climate changes and antimicrobial resistance are the most important among the complex groups of risk factors and their interactions leading to the emergence of surprisingly severe foodborne infections in humans, as it was the *E. coli* infection in 2011 in Germany. The last EARS-Net report on antibiotic resistance highlights an especially worrying situation with regard to Gram (-) bacteria, wherein one of the most emerging threats is the significantly increasing resistance to life saving antibiotics in *E. coli* and other foodborne pathogens. Additional microbiological data will reveal the importance and potential risk of zoonotic foodborne infections depending on the temperature of cultivation. Standard and modern microbiological methods and approaches will contribute to effective monitoring and evaluation of foodstuff and will complement the overall strategy to ensuring food safety and protection the consumer’s health.

FM2

METABOLIC FLUCTUATIONS IN THE METABOLISM OF PREBIOTIC OLIGOSACCHARIDES FROM LACTIC ACID BACTERIA

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Aim. The objective of this study was the characterization of the specific metabolism of different prebiotic oligosaccharides from probiotic lactic acid bacteria. To date, the majority of studies on prebiotics have focused on inulin, fructooligosaccharides (FOS), galactooligosaccharides (GalOS), and lactulose. These groups of carbohydrates are at the forefront of commercial prebio-tics due to their efficacy, testing in humans and history of safe commercial use . However, there are non-digestible oligosaccharides under investigation, such as glucooligosaccharides, xylo-oligosaccharides, gentooligosacchaides and another.

Results. The degradation and utilization of different prebiotic oligosaccharides are strain-specific and are also affected by the DP and structure of oligomers present in oligosaccharide mixture. Data obtained in the present study suggest that strains of LAB are individually able to change fermentation patterns, depending on the available substrates and metabolic pathways of the strains. The ability of different probiotic strains to metabolize prebiotic oligosaccharides depends on the efficiency of their hydrolytic systems (α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, levanase, xylanolytic enzymes ect.). The fermentation end-products obtained after cultivation in the presence of FOS, GOS, XOS and GalOS were compared to those observed on glucose; production of lactic acid was decreased, while that of acetic acid, and ethanol was increased. These results indicated that the used oligosaccharides induce LAB to form end-products of a typical mixed-acid fermentation.

Conclusions. Some speculation on the dual action of ABC system could be made in the case of FOS and XOS import and bacteriocin production in the studied strains *L. delbrueckii* B5 and *L. delbrueckii* B8. However, more studies should be performed in order to elucidate the pathways of utilization of oligosaccharides in these lactobacillus strains.

Keywords: prebiotics, oligosaccharides, lactobacillus, xylosidases, glycoside hydrolases

FM3

PROBIOTIC SOLUTION FOR VAGINAL CANDIDIASIS

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Introduction. Vaginal yeast infection, also called vaginal Candidiasis or vulvovaginal Candidiasis, is a common fungal infection of the genitals. It causes inflammation, irritation, itching, and vaginal discharge. Common Candidiasis are caused mostly by *Candida albicans.* For most infections, the treatment is an antifungal medicine (boric acid, nystatin, or flucytosine) applied inside the vagina or a single dose of fluconazole taken by mouth. Today we are looking for more harmless alternatives to these drugs and such a good solution to the problem is the application of lactobacilli, which have proven antimicrobial properties.

Aim. The aim of this study is to evaluate *in vitro* the effect of probiotic strain *Lactobacillus plantarum* GLP3 on *Candida albicans* and to estimate the efficacy of the treatment with commercially available form of GLP3 on women with proven vaginal Candidiasis.

Material and Methods. *Lactobacillus plantarum* GLP3 was screened for inhibitory activity against clinical isolate of *Candida albicans* by plate counting method. The *in vivo* effect of GLP3 was studied in a human trial with Candidiasis (+) women of different ages. Every woman was given only GLP3 in daily doses of $5.1x10^9$ CFU/g applied intravaginal for 10 days. All women were retested for a presence of *C. albicans* after the treatment.

Results. The results showed 99.8÷99.9% inhibition of the test clinical strains of *C. albicans* after 48-hours co-cultivation with *Lactobacillus plantarum* GLP3. After the intravaginal administration of GLP3 in the human trial, there was demonstrated eradication in 97% of all Candidiasis (+) women.

Conclusions*. Lactobacillus plantarum* GLP3 has *in vitro* inhibitory properties against *C. albicans*. The human trial demonstrated an effective and beneficial method of treating women of different ages with vaginal Candidiasis without the use of any antibiotics or other antimycotics with harmful side effects.

Keywords: *Lactobacillus plantarum*, *Candida albicans*, inhibition, candidiasis, treatment.

FM4

ISOLATION AND CHARACTERISATION OF LACTIC ACID BACTERIA FROM TRADITIONAL DAIRY PRODUCT KATAK

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Introduction. Katak is traditional Bulgarian fermented curd / yogurt-like product, with specific salted milk-acid taste, made from ewe's milk without starters added. Its microbiota contributes to organoleptic properties and long shelf life (up to 1 year).

Aim. The aim of the study is to investigate the autohtounous *Lactobacillus* microbiota of homemade samples of katak from Middle Stara planina region.

Methods. Decimal dilutions of katak sample were used for *Lactobacillus* isolation on De Man Rogosa and Sharpe (MRS) and Rogosa agar plates, cultivated anaerobically at 37^{o}C.

Proteolytic activity was estimated on Milk agar and Calcium caseinate agar. In addition, coagulation tests using skimmed and fresh prepared soy milk were carried out.

Antimicrobial activity against *Escherichia coli* was estimated by agar-diffusion method on MacConkey agar.

The spectrum of antimicrobial susceptibility to 16 antibiotics was characterized with Kirby–Bauer test using commercial antibiotic disks (BB-NCIPD).

Results. Microbiota of traditional katak is poorly studied. For this reason, we isolated 14 pure cultures from katak samples. They were characterized as *Lactobacillus* spp. Their species characterization, according to polyphasic taxonomy is still in progress.

Five of the strains showed fast acidification and coagulation of soy milk for up to 4 hours, along with strain-specific proteolytic activity in Milk and Calcium caseinate agar.

In vitro tests with neutralized and acid cell-free supernatants from MRS cultures showed antibacterial activity against *Escherichia coli* (HB101 and outpatient TU5 strains). All lactobacilli fulfilled EFSA's requirement for antibiotic susceptibility.

Conclusion. The autochthonous *Lactobacillus* microbiota of katak possesses biological activity and appropriate starters' characteristics.

Keywords: Lactobacilli, katak, proteolytic/antimicrobial activity

FM5

MICROBIOLOGICAL EVALUATION OF BOTTLED K-BRAND WATER

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Bottled water is widespread in schools and kindergartens, various retail outlets and partly in households. This raises the issue of water quality in bottles. Consumption of such water may be important for the health of consumers, especially for adults, young children and people with a reduced immune system.

Aim. The purpose of our study is to determine the microbiological quality of bottled water for different periods of time after bottling.

Methods. The water samples K-brandare analyzed, using membrane filtration method. The water tested has been assessed according to the indicators laid down in the Bottled Water Ordinance using standard verified methods. The studies were carried out till a year after bottling.

Results. No pathogenic microorganisms were observed.

Conclusion. The water covers the main requirements of European standards.

Keywords: bottled water, membrane filtration, assessment

FM6

DETECTION OF *PAENIBACILLUS LARVAE* SPORES IN HONEY BY CONVENTIONAL PCR AND IT'S POTENTIAL FOR AMERICAN FOULBROOT CONTROL

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Aim. The present study attempted to detect *Paenibacillus larvae* spores in naturally contaminated honeys by conventional PCR and to determine the sensitivity of the reaction with different primer pairs in order to assess its potential for American foulbrood control.

Methods. Duplicated honey samples were collected from 5 bee colonies with clinical American foulbrood and 5 clinically healthy colonies in the same apiary. The samples were analysed for the presence of *Paenibacillus larvae* spores by culture method and subsequent PCR detection in bacterial colonies.

Results. The PCR performed directly with spore DNA failed in 6 out of the 20 honeys investigated with spore load of 10 - 46 cfu/g. The established sensitivity of the reaction in the present study was 70%.

Conclusions. The adequate control of American foulbrood by analyzing of honeys for *Paenibacillus larvae* spore contamination should be done by combination of culture method followed by PCR in bacterial colonies, whose sensitivity was 100%.

Keywords: American foulbrood, *Paenibacillus larvae*, spores, honey, PCR, control

FM7

WILD GROWING HERBS - NATURAL SOURCES FOR ISOLATION OF LACTIC ACID BACTERIA

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Aim. Examination of biodiversity of lactic acid bacteria (LAB) among wild growing herbs – *Pulsatilla montana* (Hoppe) Rchb. and *Pulsatilla slaviankae* (Zimm.) Jordanov & Kozuharov in order to include them in starter communities to obtain healthy fermented milks.

Methods. The examined plants have been harvested from their natural habitats - Golo bardo mountain (*P. montana*) and Slavyanka mountain (*P. slaviankae*). Presumptive LAB have been isolated by culturing in milk and on selective media (MRS, M17, Streptococcus selective, Rogosa). Cells and colonies' morphology has been studied using microscopic analysis. To determine an affiliation of plant-derived bacteria to the group of LAB have been conducted the following tests – Gram staining, catalase assay, lactic acid synthesis, CO_2 from glucose as well as milk coagulation.

Results. Microbial isolates were obtained from different parts (flower, leaf and stem) of examined herbs and were selected based on coagulation of milk, gas formation and non-specific odour. 117 single bacterial colonies were isolated using multiple transfer on selective media. After differentiating tests were proven 73 Gram (+ve), catalase (-ve) potential LAB. Producing of lactic acid was studied. Presumptive LAB were classified as homo-/hetero fermentative, based on their ability to produce CO_2.

Conclusions. It was explored and studied the biodiversity of LAB in natural plant habitats. It has also been created a collection of LAB having a potential application in the food industry – new approach for obtaining of healthy fermented milks.

Keywords: *Pulsatilla montana* (Hoppe) Rchb., *Pulsatilla slaviankae* (Zimm.) Jordanov & Kozuharov, biodiversity, isolation, lactic acid bacteria

Acknowledgement. Grant DN 11/11, funded by the Bugarian Science Fund, Ministry of Education and Science

FM8

BAD MICROBIOTA IN FULL-CREAMED YELLOW CHEESE

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Introduction. Cheese plays significant role in human nutrition being one of most ready-to-eat food produced worldwide. Cheese is rich in microbiota – more than 400 species of LAB, Gram (+) and Gr (-) bacteria, yeasts and molds have been identified in it so far. The rich nutritional content of cheese favors also the development of foodborne pathogenic bacteria.

Aim. The aim of our study was to investigate the microbiota in commercially branded full-creamed yellow cheese with high market share. Additionally, to search for pathogenic bacteria.

Materials&Methods. 10g of yellow cheese were aseptically homogenized and 90 ml of Ringer's solution. The mixture was heated to 44^{o}C in order to reach full fat dissolution. Further 0.1 ml were plated on selective and differential media as SPS agar (*Clostridium*), MCA agar (*Staphylococcus*), KEAA (fecal enterococci), YGS (yeasts), Candida agar (*Candida* spp.), Nutrient broth (total number). The isolates were subcultured on Columbia agar with 5% sheep blood; McConkey agar (BD, USA); Saburaud and BBL CHROMagar Candida (BD, USA). Identification was carried out using Crystal (BD, USA) system; VITEK 2, BioMerieux, France, API 20 C AUX Candida (BioMerieux, France).

Results. Our results revealed that the total number of bacteria in the investigated sample was $6.9x10^5$. Cheese was negative for clostridia and coliforms but positive for yeasts – $2x10^2$ CFU/g, including *Candida* spp. - $2x10^2$ CFU/g. Annoyingly high was found the amount of fecal enterococci - $1.5x10^5$ CFU/g and staphylococci – $2x10^4$ CFU/g. Isolates of the examined bacterial groups were further identified as *Candida crusei*, *Cryptococcus neoformans*, *Enterococcus avium* and *Staphylococcus simulans.* Found at significant amount, enterococci can cause brain abscesses in humans, some of them were reported as antibiotics-resistant ones. *Enterococcus avium* is responsible for bacteremia while *Staphylococcus simulans* is associated with the endocarditis of chickens and can further be transmitted in humans through the food chain.

Conclusions. Investigated yellow cheese sample was abundant in enterococci and staphylococci, exceeding the standard. Yeasts, including *Candida* spp. responded the standard but could be harmful to immunocompromised customers or to those ones, suffering from oral diseases.

Keywords: diary products, food control, enterococci, staphylococci, *Candida*

Acknowledgement: The study was supported by Grant NBU-MF-3/2017, Faculty of Master Education, New Bulgarian University

FM9

IDENTIFICATION OF LACTOBACILLI FROM TRADITIONAL BULGARIAN WHITE-BRINED CHEESE

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Aim. Home-made dairy products are an inexhaustible source of poorly investigated nonstarter lactic acid bacteria (NSLAB), whose biodiversity and specific impact on the sensory characteristics enable its usage in the production of new functional foods. With this aim, the microflora of 17 samples of traditional Bulgarian white-brined cheese was investigated and a laboratory collection of more than 40 *Lactobacillus* ssp. was established.

Methods. The phenotypic and physiological similarity between the studied dairy lactobacilli, required the application of the polyphase taxonomy approach: (i) Biolog system – a semi-automated method for biochemical determination of *Lactobacillus* ssp.; (ii) Multiplex PCR with specific primers for the differentiation of closely related species from the group of *Lactobacillus plantarum;* (iii) 16S rDNA analysis – the golden standard in bacterial identification.

Results. The investigated strains have been identified as: *L. plantarum* (13 strains), *Lactobacillus fermentum* (2 strains), *Lactobacillus delbrueckii* spp*. lactis* (1 strain) and *Lactobacillus salivarius* (1 strain).

Conclusions. The strain-specific character of probiotics requires the investigation of bacterial genome and the use of molecular methods that can detect even the smallest differences in species and even between strains for further application in dairy industry.

Keywords: *Lactobacillus* ssp.*,* Multiplex PCR, RAPD PCR

FM10

UTILIZATION OF HUMAN BREAST MILK OLIGOSACCHARIDES FROM LACTIC ACID BACTERIA STRAINS ISOLATED FROM NEONATAL GUT MICROBIOTA

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Aim. The purpose of this study is, characterization of metabolism of lactic acid bacteria, isolated from human breast milk cultivated in media with human milk oligosaccharides

Methods. Breast milk was collected from 20 healthy women aged between 23 and 35 years who were breastfeeding children from birth to 1 years old. A total of 150 isolates were obtained by plating 100µl of the milk on non-selective MRS agar broth. Bacterial genomic DNA was extracted using Qiagen DNA axtraction kit(QIA amp, Germany). The DNA was used for PCR amplification of 16S ribosomal DNA. The purified PCR product of each isolate were sequenced by Macrogen, Holland. Sequences of all isolates from each were BLAST with a reference sequence for comparision.

Results. The human breast milk continuous supply of commensal, mutualistic and potentially probiotic bacteria to the infant gut. 30 isolates were identified in this study and their metabolic profile showed that all of the isolated were able to metabolise a wide variety of carbon sources including HMO and the ability to survive in many environments. Data obtained in the present study suggest that isolated strains of *Lactobacillus fermentum* are individually able to metabolize sugars in human breast milk using different carbohydrate enzymes - α-galactosidase, β-galactosidase, fucosyltransferase.

Conclusions. The in vitro studies described in this paper suggest that HMOS from s human breast milk have potential to activated *L. fermentum* bacteria isolated from human breast milk and are worthy of further in vivo study.

Keywords: human breast milk, *Lactobacillus fermentum*., probiotic, oligosaccharides, HMOS

Veterinary Microbiology and Zoonozes

VMZ1

PEST OF SMALL RUMINANTS (PESTE DES PETITS RUMINANTS)

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The pest of small ruminants is an acute-onset, highly contagious disease of sheep and goats. It is caused by the peste des petits ruminants virus (PPRV) belonging to the genus *Morbillivirus* within the family *Paramyxoviridae*. The epizootiological features of the disease are characterized by transmission of infection through direct contact between a healthy and an infected animal. Other modes of transmission, such as vector transmission, are ruled out. Infections caused by wild animals have not been detected either, because of the brief exposure to such animals. The virus is unstable and dies in the external environment within a few days. It is excreted by the infected animal as early as the incubation period, which lasts from 5 to 10 days, but the peak period of virus excretion occurs during the acute stage of clinical manifestation. Contact animals become infected by exhaled aerosols, conjunctival and nasal discharge, and diarrheal faeces of infected animals. As a result, the disease spreads rapidly within the flock, with a large number of animals being infected within a short period of time. Surviving animals are virus-free, therefore there is no virus carriage.

The clinical presentation is severe and includes conjunctivitis with mucopurulent discharge, discharge from the nasal passages, necrosis and ulcers in the oral cavity and digestive system organs, bronchopneumonia. The duration of the disease is a few days, typically ending with death. Mortality is high and reaches 90% in young animals and goats. Treatment is not applied. In the country where the disease is first established, all animals in the affected flock are destroyed. The rest of the flocks are quarantined in spatial isolation and kept under strict surveillance for at least 20 days after the last case of the disease. Diagnostic tests are performed on blood samples to detect the virus or antibodies against it. Specimens for virological examination are collected from secretions and excretions. Tissue samples from internal organs are taken only from fresh cadavers. During transportation, samples must be kept refrigerated or frozen.

In countries where the disease appears to be stationary, immunoprophylaxis is carried out with vaccines prepared from attenuated cattle-plague virus because of the antigenic and immunogenic similarity between these two viruses, or with attenuated PPRV virus.

To exclude cross-border transmission of the disease, close cooperation must exist between the country at risk of infection and the country affected by the disease.

VMZ2

STUDIES OF MOLECULAR BIOLOGICAL PECULIARITY OF BOVINE HERPESVIRUS 4 (BHV 4)

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Studies of the molecular biological features of isolated in Bulgaria bovine herpesvirus 4 (BHV 4) have been conducted. Two types of polymerase chain reaction (PCR) have been developed and applied to demonstrate the gB and TK genes of the virus. By using this two PCR the isolated strains are confirmed as BHV 4. Both methods can be used successfully for the rapid diagnosis of BHV 4 infections.

For study the molecular-biological peculiarities of isolated strains of BHV 4, a restriction enzyme analysis was performed using various types of restriction enzymes. The tested Bulgarian strains differed in their restrictase genomic profile from the reference European strain "Movar 33/63" and from the American strain "DN 599", and show a clear difference between each other. No clear correlation has been established between the restriction enzyme profiles and the tropism of the isolated viruses.

Sequencing of isolated and typified BHV 4 strains was performed and found to have sequenced homology with the reference European strain "Movar 33/63". After construction of the phylogenetic tree, strains “Levski”, “Nikolovo” and “Svoboda” were alight in one branch of the phylogenetic tree, while the “Kazichene” and “Godech” strains in the same branch with the reference strain "Movar 33/63”.

Applied molecular biology methods can be successfully used for differentiation and detailed genetic characterization of the isolated BHV 4 strains.

VMZ3

20 YEARS EPIDEMIOLOGICAL, EPIZOOTICAL AND PHYLOGENETIC INVESTIGATIONS ON TULAREMIA IN BULGARIA

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Aim. Tularemia has re-emerged in Bulgaria since 1998 after 30 years of epidemiological silence. The aim of this report was to summarize the results of studies on tularemia in the last 20 years in Bulgaria. In addition, we reported results on genotyping of strains isolated during epizooty among hares in 2014-2015.

Methods. The isolation and characterization of *Francisella tularensis* strains were performed by classical microbiological methods. The genotyping of *F. tularensis* isolated by hares was performed by polymerase chain reaction (PCR) and single-nucleotide polymorphism analysis (SNP).

Results. The oropharyngeal form of tularemia predominates in man and typhoidal in brown hare. The strains isolated showed high phenotypic and genetic diversity and common pattern of antibiotic susceptibility. The genetic analysis showed that the outbreaks in the recent years as well as in the 1960s involved multiple clones and new genetic diversity.

Conclusions. The small genetic difference found between some strains from both old and recent outbreaks indicates that *F. tularensis* is able to persist locally over long time periods without causing outbreaks.

Keywords: *Francisella tularensis*, tularemia, man, brown hare, PCR, SNP

VMZ4

CHLAMYDIA ABORTUS AND *COXIELLA BURNETII* RELATED ABORTIONS IN SMALL RUMINANTS IN BULGARIA DURING A FIVE YEARS PERIOD (2013-2018)

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The results from the polymerase chain reaction (PCR) and serological testing for *Chlamydia abortus* and *Coxiella burnetii* of abortion submissions (placentae or foetal organ samples) and sera from 56 sheep and 54 goats are presented. The samples were submitted for routine diagnostic procedures at the Laboratory for Chlamydial and Rickettsial diseases, NDRVMI during a five years period (2013-2018). Samples from 48 sheep and 28 goats were tested by conventional PCR for the presence of one or both of infections, while other 34 small ruminants were investigated only serologically by ELISA. In some cases one and the same animal was tested both serologically and by PCR. From 43 ovine and 24 caprine samples tested by PCR for *C. abortus*, 24 (35.8%) resulted positive, respectively 18 (41.9%) at sheep and 6 (25%) in goats.. Five of the 12 PCR-tested goat samples were positive for *C. burnetii*, whereas no sample of 17 sheep tested showed presence of this agent. Antibodies against these two intracellular bacteria were detected in 20 (23%) out of 87 sera of aborted animals, suggesting, or confirming their probable etiological role. The results show that *C. abortus* plays a leading role as a cause of the abortions in small ruminants as a whole, but *C. burnetii* also should not be ignored, especially presuming it's zoonotic potential.

Keywords: *Chlamydia abortus*, *Coxiella burnetii*, small ruminants, abortions

VMZ5

EFFECT OF *STAPHYLOCOCCUS AUREUS* INFECTION ON THE ACTIVITIES OF THE ALCALINE PHOSPHATASE, ASPARTATE AND ALANINE TRANSFERASES IN THE BLOOD PLASMA OF RABBITS

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Aim. The aim of this paper is to investigate the dynamics of the activities of alkaline phosphatase (AP), aspartate (ASAT) and alanine (ALAT) transferases in plasma of rabbits that are infected with *Staphylococcus aureus*.

Methods. The experiment was carried out with 12 male rabbits (White New Zealand), divided into two groups: experimental and control. Blood samples from each rabbit were taken before the euthanasia of *v. auricularis externa* as follows: at 3 months of age, coinciding with 0 h before infection, and at 6, 24, 48, 72 h and on days 7, 14, and 21 after *S. aureus* infection in sterile heparinized tubes. The AP, ASAT and ALAT were performed on a semi-automated biochemical analyzer, Section in Biochemistry, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria.

Results. The results showed significant decreasing ($P < 0.05$) of AP on day 14 comparing to the control group ($P<0,01$), significant increasing of ALAT ($P<0,01$) comparing to the control group and significant increasing of ASAT on day 14 and day 21 in the experimental group comparing initial level.

Keywords: *Staphylococcus aureus,* alkaline phosphatase (AP), aspartate (ASAT), alanine (ALAT) transferases, rabbits

VMZ6

COMPARATIVE STUDY OF THE EFFECT OF ELECTROCHEMICALLY ACTIVATED WATER SOLUTIONS ON *PSEUDOMONAS AERUGINOSA*

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Aim. Studies have been carried out to determine the sensitivity of *Pseudomonas aeruginosa* to activated aqueous solutions (anolytes and catholytes) *in vitro*.

Methods. The solutions were obtained by electrochemical activation of water with 0.8% NaCl, as well as 0.4% NaCl and 0.4% Na_2CO_3 and tested at final concentrations of 50% and 100%. The disinfectant Virkon S was used as a control. After different intervals of action of the solutions on a suspensions of *P. aeruginosa* at a final concentrations of 10^6 cells/ml were seeded on Cetrimide agar and cultured for 24 hours at 37° C.

Results. An inhibitory effect on *P. aeruginosa* was established depending on the type and final concentration of the activated solutions.

Conclusions. Electrochemically activated aqueous solutions with 0.4% NaCl and 0.4% Na_2CO_3 exert inhibitory action on a *P. aeruginosa* suspension at concentration of 10^6 cells/ml.

Keywords: anolyte, catholyte, *Pseudomonas aeruginosa*, antibacterial activity

VMZ7

PRELIMINARY INSIGHT INTO GENETIC DIVERSITY OF *MYCOBACTERIUM BOVIS* STRAINS IN CATTLE IN BULGARIA

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Aim. Bovine tuberculosis caused by *Mycobacterium bovis* is major problem for cattle with annual economic losses to stock-raising. From 2000 to 2015 the disease shows cyclicity in dairy farms in different regions of Bulgaria. The lack of information on the genetic diversity of *M. bovis* on the territory of country and the frequency of distribution of different genotypes makes it necessary to define the molecular characterization of isolated strains.

Methods. Macroscopic and microscopic observation of 35 lymph node materials from slaughtered cattle, received in the National Reference Laboratory of Animal Tuberculosis, RD4-PCR, spoligotyping.

Results. In 27 of the examined lymph nodes we found specific lesions for bovine tuberculosis. The findings were confirmed bacteriologically and by convectional PCR. To differentiate *M. bovis* from other *M. tubrculosis* complex subtypes, we used primers flanking specific deletion (RD4) in the genome of *M. bovis* and received the 446 bp DNA product. The spoligotyping was subdivided the strains into 12 spoligotypes: 2 unique profiles and 10 profiles shared by 2 to 25 strains. SB1176 was the dominant spoligotype followed by SB0133. New spoligotypes had been reported to the *M. bovis* database.

Conclusions. The application of molecular-genetic methods for genotyping of *M. bovis* strains such as RD4-PCR and spoligotyping, will initiate new possibilities to tracking the transmission of *M. bovis*. Further molecular investigations of *M. bovis* strains are needed to characterize the genetic diversity and population structure of *M. bovis* strains isolated from cattle in Bulgaria.

Keywords: *Mycobacterium bovis*, RD4-PCR, spoligotyping, Bulgaria

Acknowledgements: This work was supported by the grant DN16/12 of the National Science Fund, Bulgaria

VMZ8

OUTBREAK OF *AEROMONAS HYDROPHILA* ASSOCIATED WITH THE PARASITIC INFECTION *ICHTHYOPHTHIRIUS MULTIFILIIS* IN EUROPEAN CATFISH (*SILURUS GLANIS*) JUVENILES

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Outbreak of a disease observed on European catfish (*Silurus glanis*) juveniles 8-10 cm long manifested by white nodules and spots mainly on the head and hemorrhages in the skin occurred on June, 2018. The outbreak was investigated by using a combination of methods that included clinical observations, gross and pathology examination and bacterial isolation. Co-infection of *Aeromonas hydrophila* and *Ichtyophthirius multifiliis* a holotrichous ciliate, was found to be the cause of the outbreak. In order to control the outbreak, the fish density was reduced and the fish were treated with commercial chemical Anti-spot according to the manufacturer instructions. Susceptibility of the isolated *A. hydrophila* strain to the commercial chemical Anti- spot and different antibiotics was determined.

Keywords: European catfish (*Silurus glanis*), co-infection, *Aeromonas hydrophila, Ichtyophthirius multifiliis*

VMZ9

DETERMINATION OF PROTEINS IN BOVINE AND AVIUM PPD TUBERCULIN

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Introduction. Tuberculin skin test is one of the oldest and widely used *in vivo* methods for detection of *Mycobacterium bovis* infections in cattle. Purified protein derivative (PPD) a mixture of antigens containing proteins, peptides, nucleic acids and polysaccharides. It is assumed that biological activity and specificity of PPD is due to its protein content. The main objective of this project is to obtain a basic information about the composition of *M.bovis* PPD and *M.avium* PPD, produced in Bulgaria and to develop suitable critearia for establishing quality control procedures that will indicate the purity of these products.

Method. The total nitrogen content of tuberculins was determined using micro-Kjeldahl procedure.

Results. The protein content of samples corresponded to the observed amount of nitrogen. The average loss of protein was different at various stages of production reaching highest levels during the sterile filtration (25-30%). However, protein content of 0.98 mg/ml was determined after filtration in a sample with concentration 1 mg/ml, compared to unfiltered product where average protein content was 0.80 mg/ml. When dry tuberculin was examined samples were diluted in order to avoid foaming and thus facilitate complete digestion of tuberculins during analyses.

Conclusion. The analysis of liquid and dry unfiltered bovine and avian tuberculins compared to filtrated product shows that filtration stage is critical for the specificity and antigenic activity of PPD. The protein loss is around 20%. The variations of loss may be due to production process, preparation of samples during analyses or the varying amount of tuberculin present in the culture filtrates.

VMZ10

PREVALENCE OF YERSINIA ENTEROCOLITICA IN FATTENING PIGS ORIGINATED FROM DIFFERENT REGIONS OF BULGARIA

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Aim. Since 1960s*, Yersinia enterocolitica* has been identified as an important cause of yersiniosis in humans. Sporadic cases in Europe are often related with consummation of pork meat. Pigs are identified as asymptomatic carriers of human pathogenic strains, especially biotype 4 serotype O:3. The main object of this study was to provide data about the prevalence of human pathogenic *Y. enterocolitica* strains in slaughtered aged pigs in Bulgaria and their regional distribution.

Methods. Samples of pig tonsils and feces were collected from seven farms belonging to four different regions. *Y. enterocolitica* was isolated using PSB broth enrichment and CIN agar culture according to ISO 10273-2003. Colonies were identified using bio-serotyping methods, PCR assay and Loop Mediated DNA Amplification (LAMP).

Results. Of examined 600 pigs 907 bull's eye colonies were isolated on CIN agar and 215 have shown to be *Y. enterocolitica* according biochemical identification protocol. The prevalence of pathogenic *Y. enterocolitica* was in total 6,7% and ranged between 1 and 13,7% among different farms. The *ail* gene has be found in 42 isolates. The presence of *Y. enterocolitica* strains was confirmed by the LAMP method based on the identification of the *phoP*[*] gene.

Conclusions. Our study demonstrates that significant number of pigs carry pathogenic *Y. enterocolitica* strains in their tonsils, being potential risk for contamination of pork and the health of consumers. The LAMP method is sensitive and reliable; thus, representing a promising perspective for fast identification of pathogenic *Y. enterocolitica* in conjunction with other tools.

Keywords: *Yersinia enterocolitica*, LAMP, *ail* gene

*Li Y et al., *Mol Cellular Probes* 24 (2010) 68–71

Varia

Var1

THE GLYCONOSIDE MYCONOSIDE, EXTRACTED FROM *HABERLEA RHODOPENSIS* IS A PROMISING AGENT FOR PHYTOPHARMACOLOGICAL DEVELOPMENT OF BALANCED CYTOTOXICITY

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Aim. The aim of present study was to investigate the antioxidant properties of myconoside, extracted from *Haberlea rhodopensis* and its effect on epithelial cancer cells.

Methods. Antioxidant activity was assayed using DPPH and NBT methods. Cytotoxic and morphological investigations were performed by crystal violet method. Lipid peroxidation and protein damage were evaluated using MDA Assay Kit and Sohal et al. (1994) method, respectively.

Results. DPPH and NBT methods confirmed the high free radical scavenging activity of myconoside.

We studied IC_{50} of the metabolite on normal (MDCK) and cancer cells (A549). The morphological investigations demonstrated strong cytotoxic effect on cancer cells at concentrations above 3 μg/ml while MDCK cells were damaged by concentrations above 7.5 μg/ml. Treated cells disrupts the typical form of epithelial cells morphology; some of the cells are rounded off and cease to perform their functions. Lipid peroxidation and protein damage confirmed the cytotoxic effect of myconoside especially on cancer epithelial cells.

Conclusions. Myconoside, extracted from *Haberlea rhodopensis* is destroying selectively cancer cells at low concentrations. This, together with the strong antioxidant activity, makes it a promising agent for phytopharmacological development in search of balanced cytotoxicity.

Keywords: Myconoside, *Haberlea rhodopensis*, cytotoxicity, antioxidant activity, cancer epithelial cells.

FEMS & MedILS postdocs summer school

NEW OPPORTUNITIES AND CHALLENGES FOR EXCELLENT POSTDOC MICROBIOLOGISTS

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[2]FEMS Director of Events and Internationalization

Starting in 2019, FEMS, in collaboration with MedILS (Mediterranean Institute for Life sciences) from Split, Croatia, will organize serial of FEMS postdoc summer schools, for excellent European and non-European postdocs, managing by worldwide scientifically recognized and distinguish professors, lecturers, researchers and scientists. It would be a new landmark of FEMS, a place for connection of young and senior scientists, knowledge exchange, and production of ideas for new scientific projects.

This common project with enormous potential for benefit of microbiology and scientific community worldwide is based on missions and visions of FEMS and MedILS, to advance microbiology, spread scientific knowledge and innovation, maximise communication, teaching, learning, brainstorming and implementing the world's highest standards of scientific work, style and ethics.

MedILS was founded in 2003, as a nonprofit research institute, to be a leading international center of excellence in the field of natural sciences. Institute is situated in pine forest in the heart of hill Marjan (178 m).

Split is second largest city in Croatia, largest in Dalmatia, historic, cultural and trade Center with borderline subtropical and Mediterranean climate with hot moderately dry summers. Population about 180.000 inhabitants. University of Split founded in 1974, consist of 12 faculties and 26.000 students.

Great connections of Split are provided by international airport and excellent highway.

Institute located in a beautiful completely renovated building, comprises conference hall for 170 people, several classrooms for smaller groups, and complete audiovisual equipment.

Several equipped lab are on disposal for experiments for postdocs, depending on the school program.

Accommodation at MedILS is sufficient to accommodate 10 teachers and 25 students.

Duration of the summer school – 10 days, last week of august and first week of September 2019.

First director of the FEMS postdoc summer school is Prof. D-r Miroslav Radman who is co-founder and Scientific Director of MedILS. He is great, worldwide known scientist, who received lot of awards, such is FEMS Lwoff award in 2013. He is a member of EAM (European Academy of Microbioogy), Member of French Academy of Sciences, Member of Croatian Academy of Arts and Sciences, Foreign honorary member of American Academy of Arts and Sciences.

Now he is identifying co-director of the school and worldwide distinguish scientists and experts to be teachers and mentors.

He proposed the title (Biological Robustness: Evolution of Bacterial Resistance to Death) and prepared the program of the first FEMS postdoc summer school.

All information will be available very soon on FEMS web: www.fems-microbiology.org

Acknowledgment to the Croatian Microbiological Society (prof. Danko Hajsig-former President and former delegate in FEMS, prof. Roberto Antolovic current President, and d-r Duska Vujalkija) for their help and proposal MedILS as a venue for the FEMS postdoc summer school.

MINI SYMPOSIUM AQUACHIM

Determination of cellular metabolism and sensibility to chemical agents

1. **Development of novel target antimicrobial agents**
2. **Optimization of bacterial lines and conditions of cultivation at biotechnological industry**
3. **Characterization of phenotypes for taxonomical en epidemiological investigations**

BIOCOM-TRENDAFILOV PRESENTATION

FAST AND RELIABLE METHODS FOR MICROBIOLOGICAL CONTROL OF SURFACES, FOOD AND BEVERAGES

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MINI SYMPOSIUM ELTA 90M

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Halina Panicharova

Microbiology sales expert

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