BULGARIAN ACADEMY OF SCIENCES

70 YEARS

THE STEPHAN ANGELOFF INSTITUTE OF MICROBIOLOGY - BAS

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INTERNATIONAL SCIENTIFIC CONFERENCE

MICROBIOLOGY FOR A BETTER HEALTH AND INDUSTRY

PROGRAM AND ABSTRACT BOOK

March 14-15, 2017

The Grand Hall of BAS

1, "November15" str., Sofia

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Tuesday, March 14th, 2017

Opening Ceremony

10.00 - 10.30 Musical greetings

10.30 - 11.00 Official speech of the Director of the Stephan Angeloff Institute of Microbiology

11.00 - 11.30 Congratulatory addresses

11.30 – 12.00 Awarding the best scientific work of young microbiologist by the Foundation "Acad. Prof. Dr. Stefan Angelov"

12.00 – 13.00 Lunch break

13.00 – 14.00 Registration

Scientific session I

Plenary lectures

Chairpersons: Hristo Najdenski
Elisabeth Carniel

14.00 - 14.30 Is the plague really an old disease?
Elisabeth Carniel, Institut Pasteur, Paris

14.30 - 15.00 Oncolytic virus based cancer therapy
Jean Rommelaere, Assia Angelova, German Cancer Research Centre

15.00 - 15.30 Novel concepts for sustainable food production and challenges in relation to food safety
Lieve Herman, ILVO, Belgium

15.30 - 16.00 Coffee break

Presentation of the company "Ridacom"

16.00 – 16.30 Extremophiles in Astrobiology
Paola Di Donato, Annarita Poli, Ida Romano, Barbara Nicolaus, Institute of Biomolecular Chemistry, Naples, Italy

16.30 - 16.50 Horizon 2020 and the incentives programs with a focus on Bulgaria
David Itier, Institut Pasteur, Paris

Poster session I – P1 - P14

Chairpersons: Lyubka Doumanova
Stoyanka Stoitsova

16.50 – 17.30 Poster presentation and discussion

19.30 – 22.00 Gala dinner

Restaurant in "Dom na ucheniya"
50, "Shipchensky prohod" Bld., Sofia 1113

Wednesday, March 15th, 2017

Scientific session II

Chairpersons: Angel S. Galabov
Milton S. da Costa

Plenary lectures

9.00 - 9.30 Osmolytes from mesophiles to hyperthermophiles and back
Milton S. da Costa, Universidade de Coimbra, Portugal

9.30 – 10.00 Harnessing benefit from targeting tumor associated carbohydrate antigens
Tomas Kieber-Emmons, University of Arkansas, USA

10.00-10.30 Neopterin production and tryptophan breakdown in infectious diseases
Dietmar Fuchs, University of Insbruck Austria

10.30 - 11.00 Coffee break
Presentation of the company "Ridacom"

11.00 - 11.30 New facets in the mechanism of action of the alkylphosphocholine erufosine
Martin Berger, German Cancer Research Center, Heidelberg, Germany

11.30 – 11.50 "One Health" approach toward antimicrobial resistance
Gabriel Ionescu, "Carol Davila" University of Medicine and Pharmacy, "Cantacuzino" National Institute of Research, Bucharest, Romania

11.50 – 12.10 Control of enterovirus circulation in the post-certification period of polio eradication
Nataliya Romanenkova, Institut Pasteur, Saint-Petersburg, Russia

12.10 – 12.30 Pasteur Institute in Novi Sad, Serbia, 95 years on Rabies Prophylaxis
Dušan Lalošević, Pasteur Institute of Novi Sad, Serbia

12.30 – 14.00 Lunch break

Scientific session III

Chairpersons: Lyudmila Kabaivanova

Milka Mileva

Plenary lecture

14.00 – 14.30 Epidemic resistant genotypes of *Mycobacterium tuberculosis* emerging in Russia and former Soviet Union countries

Igor Mokrousov, St. Petersburg Pasteur Institute

Oral presentations

14.30 – 14.40 S-oxygenation of dithiocarbonic acid derivatives (xanthates) by flavoprotein monooxygenases as the primary mechanism of their antimycobacterial activity

Stanislav Yanev[1], Tsveta Stoyanova[1], Violeta Valcheva[2], Hristo Najdenski[2]

[1]Institute of Neurobiology, [2]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

14.40 – 14.50 From laboratory to the plant. Specific requirements and attempt for efficient industrial application of fermentation technologies

Ratkov A., Dimov I., Kristeva J., Filipov F., Petkova R., The Stephan Angeloff Institute of Microbiology

14.50 – 15.00 Influence of low temperature on the degradation ability of fungal strains isolated from Antarctic soils

Maria Gerginova, Katya Litova, Nadejda Peneva, Zlatka Alexieva, The Stephan Angeloff Institute of Microbiology

15.00 – 15.10 Beneficial microbiota of traditional Bulgarian dairy product

Danova S[1], Nemska V[1,2] [1]Department of Microbial Genetics, The Stephan Angeloff Institute of Microbiology, [2]University of Chemical Technology and Metallurgy

15.10 – 15.20 Posttranscriptional gene silencing as a tool to control Coxsackievirus infections

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

[1]Institute of Soil Science, Agrotechnologies and Plant Protection "N. Pushkarov"

[2]The Stephan Angeloff Institute of Microbiology

15.20– 15.40 Coffee break

Presentation of the company “Ridacom"

Scientific session IV

Chairpersons: Margarita Kamburova

Svetla Danova

Oral presentations

15.40 - 15.50 Anaerobic biodegradation of lignocellulosic wastes for biohydrogen and biomethane production-study and mathematical modeling
Venelin Hubenov, Lyudmila Kabaivanova, Dencho Denchev, Snejanka Mihaylova, Vasil Lakov, Elena Chorukova, Ivan Simeonov, The Stephan Angeloff Institute of Microbiology

15.50 – 16.00 Global analysis of CO_2 fixation system from flue gas in photobioreactors (PBRs) by using microalgae
Alexander Kroumov, The Stephan Angeloff Institute of Microbiology

16.00 – 16.10 Antimicrobial and cytotoxic potential of extracts and compounds derived from the plant Geum urbanum L.
Lyudmila Dimitrova, Maya Zaharieva, M. Popova, Iva Tsvetkova, S. Konstantinov, Vasya Bankova, Hristo Najdenski, The Stephan Angeloff Institute of Microbiology

Poster session II - P15 – P32

Chairpersons: Milka Mileva

Anastas Pashov

16.10 – 16.50 Poster presentation and discussion

16.50 Closing remarks

ABSTRACTS

Tuesday, March 14th, 2017

Scientific session I
Plenary lectures

14.00 – 14.30 IS THE PLAGUE REALLY AN OLD DISEASE?

PL 1 Elisabeth Carniel

Yersinia Research Unit, National Reference Laboratory and WHO Collaborating Center for Yersinia, Institut Pasteur, 28, rue du Dr. Roux, 75724 Paris Cedex 15, France

The plague is a highly severe and often-fatal disease that has left indelible marks in the collective unconscious. Since the Christian era, three pandemics were attributed to the plague bacillus, *Yersinia pestis*. There is no doubt that the third pandemic, which started from Hong-Kong in 1894, was caused by this bacillus. However, due to inconsistencies between the clinical and epidemiological characteristics of plague reported in historical records and in modern times, there was a debate regarding whether *Y. pestis* was the causative agent of the first (Justinian plague, 6th century) and second (Black Death, 14th-18th) pandemics. The development of paleomicrobiology has made possible to revisit plague pandemics and the microevolution of *Y. pestis*. Here we will give a brief overview of the current status of the plague today and we will present our current knowledge about the origin of the plague bacillus, its place of emergence, and its age.

14.30 - 15.00 ONCOLYTIC VIRUS BASED CANCER THERAPY

PL 2 Jean Rommelaere, Assja Angelova

German Cancer Research Center (DKFZ), Heidelberg (DE)

Oncolytic virotherapy is a novel emerging modality of cancer treatment. Oncolytic viruses under current investigation include members of the species "rodent protoparvovirus 1". These viruses consist of an icosahedral protein capsid about 25nm in diameter, containing a linear single-stranded DNA genome of approximately 5000 nucleotides. Their genome comprises two tanscription units encoding nonstructural and capsid proteins, whose integrity is important for viral infectiousness. This presentation focuses on the oncolytic parvovirus H-1PV, whose natural host is the rat but which can replicate in and kill a number of tumor-derived human cells, while sparing their normal counterparts. As H-1PV also exerts tumor-suppressive action in various animal models, it is being assessed and developed as a potential tool for cancer therapy and/or prevention. H-1PV antitumor action has two components: direct oncotoxicity and anticancer immunostimulation. The latter adjuvant effect depends, at least in part, on the immunogenicity of viral oncolysates, and is instrumental in mediating virus-induced anticancer vaccination in animal models. The duality of H-1PV activity will be discussed in the context of a recent clinical study showing the safety and surrogate efficacy of this virus in patients afflicted with recurrent glioblastoma.

15.00 – 15.30 NOVEL CONCEPTS FOR SUSTAINABLE FOOD PRODUCTION AND
PL 3 CHALLENGES IN RELATION TO FOOD SAFETY

Lieve Herman

ILVO, Flanders Research Institute for Agriculture, Fisheries and Food, Belgium

Sustainable food production should be nutritious and healthy, should have a limited impact on the environment, should be economically fair and affordable and socio-culturally acceptable. To balance

all these demands, it is necessary to develop innovative concepts and to adapt current reference frameworks. Research is concentrated on: 1) the optimal valorization of biomasses e.g. to packaging material and the reduction of rest streams; 2) the replacement of meat by alternative protein sources as insects, mycoproteins, extended meat products; 3) the reduction of antimicrobial pressure in the environment due to animal production by mitigation of manure management in relation to environmental stability of antimicrobials, antimicrobial resistant bacteria, and antimicrobial resistance genes and by decreasing antibiotic use by improving animal health; 4) the Improvement of food safety by reducing shedding zoonotic pathogens e.g. the use of organic acids to reduce *Salmonella* shedding in pigs and the use of botanic compounds and probiotics to reduce *Campylobacter* shedding in chickens; 5) taste and nutrients in relation to a balanced human diet e.g. by minimal food processing and new product formulations; 6) the optimal use of natural resources e.g. the use of clean water. Implementing these innovative concepts requires special attention on food safety because 'Food which is not safe cannot be considered as food'.

16.00 – 16.30 EXTREMOPHILES IN ASTROBIOLOGY

PL 4 Di Donato P.[1,2], Romano I.[1], Poli A.[1], Nicolaus B.[1]

[1]*Consiglio Nazionale delle Ricerche (C.N.R.), Institute of Biomolecular Chemistry ICB-CNR, Via Campi Flegrei, 34, 80078, Pozzuoli, Naples, Italy*
[2]*Department of Science and Technology, University of Naples "Parthenope", Centro Direzionale, Isola C4, 80143, Naples, Italy*

Astrobiology is a very recent discipline that has been accepted only in 1979 by the International Astronomical Union (IAU). Astrobiology addresses the question of the origin, the evolution and distribution of life in the universe; it explores the limits at which life can occur and its tasks include the search for life and habitable locations outside Earth.
The study of Extremophiles, i.e. those microorganisms that are able to live and thrive on Earth in several extreme environments, is significantly relevant in Astrobiology. Indeed the study of the origin of life and the search for extraterrestrial life are encouraged by the consideration that the environmental conditions in space and in other Solar System bodies are closely similar to those of severe environments on Earth in which Extremophiles can be found.
For such reasons Extremophiles are considered as valuable biological models for Astrobiology studies, as confirmed by recent findings that confirmed that these organisms could resist to the interstellar transport from or to Earth, and that they could be able to proliferate also in extreme conditions mimicking the space environments.

Poster session I
16.50 – 17.30 Poster presentation and discussion

P1 GROWTH CONDITIONS AND BIOCHEMICAL CHARACTERISTICS OF TWO RED MICROALGAL STRAINS

Ivanova J.[1], Kabaivanova L.[2]

[1] Institute of Plant Physiology and Genetics, Department Experimental algology, Bulgarian Academy of Sciences,
[2] Stephan Angeloff Institute of Microbiology, Department Applied Microbiology, Bulgarian Academy of Sciences,

Algae have received increased attention over recent years for many biotechnological applications based on their ability to use photosynthesis for biomass production and possibilities of increased production capacities. They are sources of highly active secondary metabolites that can find different applications. Two strains of red microalgae *Rhodella reticulata* and *Porphyridium cruentum* were involved in the experiments. Growth and exoheteropolysaccharide production by the strains were estimated and compared. Growth of *Rhodella reticulata* started more rapidly but at the 72th hour reached the stationary phase. For *Porphyridium cruentum* stationary state was at the 96th hour but the accumulated biomass was greater in quantity at the 144th hour (1.28 -fold higher). The viscosity showing the amount of extracellular polysaccharide was 4.6 mPa.s for *Rhodella reticulata* compared to 5.4 mPa.s for *Porphyridium cruentum* measured at the144th hour, which is 1.2 fold higher. The biochemical composition characteristics of both investigated strains were studied and showed similar composition.
Key words: *Rhodella reticulata, Porphyridium cruentum*, growth conditions, biochemical characteristics

P 2 VARIABILITY OF THE SURFACE GLYCOME AS AN ADAPTATION IN HOST-PATHOGEN INTERACTIONS

Paunova-Krasteva, T.[1], Georgieva, K.[2], Marhova, M.[3], Borisova, D.[1], Stoitsova, S.[1]

[1]Department of General Microbiology, Section "Morphology of Microorganisms and Electron Microscopy, The Stephan Angeloff Institute of Microbiology, BAS;
[2]Department of Animal Diversity and Resources, Ultrastructure of Parasites RG, Institute of Biodiversity and Ecosystem Research, BAS;
[3]Department "Biochemistry and Microbiology", Faculty of Biology, Plovdiv University "Paisii Hilendarski"

The surfaces of cells from all domains of life are characterised by the presence of glycoconjugates (glycoproteins or glycolipids). They are an essential participant in cell-to-cell interactions, including the host-invader interplay. In host-pathogen interaction, glycoconjugates represent the major class of pathogen-associated molecular patterns (PAMPs), which is recognised by host patern-recognition molecules. One large class of patern recognition molecules is the carbohydrate-binding proteins, or lectins, spread from viruses through all microbe, plant and animal taxa. Lectins with defined sugar specificities are on the other hand a wide-spread tool for the analysis of cellular glycoconjugates. In this review we summarise the experience of our team in characterising the surface glycome of bacteria and parasitic flatworms by lectinology techniques. In addition we included some reference data on viruses and protozoa. Concerning bacteria, we noted significant difference of the density of exposed lectin-binding epitopes between otherwise genetically identical bacteria. Within the bacterial population, the balance between cells with different degree of surface exposure of lectin-binding sites

can be shifted dependent on growth conditions, and determines a variable degree of recognition by host lectins. In cestodes, regional variation of lectin binding has been observed along the strobila. Trematodes are characterised by the capacity to vary their surface glycome in relation to the developmental stage. Thus, the phenomenon of variation of the surface sugar coating is ubiquitous in pro- and eukaryotic pathogens, which underlines its importance in host-pathogen interactions.
Key words: surface glycome variability, bacteria, flatworms

P 3 DETECTION OF ORAL HUMAN PAPILLOMAVIRUSES (HPV)

Grozdanov, P., Simeonova, L., Nikolova, I., Galabov, A.S.

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

Squamous cell carcinomas (SCCs) are the most common malignancy in the head and neck region. Common risk factors in head and neck squamous cell carcinoma (HNSCC) are smoking and alcohol abuse, however, in an increasing proportion of cases, no significant smoking or drinking history has been reported.
Approximately 35 years ago, a role of human papillomavirus (HPV) in cervical cancer was postulated. Today, it is well established how this very heterogeneous virus family represents an important human carcinogen, causing not only the vast majority of cervical and ano-genital tumors, but also a variable number of cancers in other districts of the human body including the head and neck. The fifth leading cause of cancer mortality rate proved oropharyngeal carcinoma (OPSCC) - throat cancer associated with positive results for the presence of HPV. HPV oral and oropharygeal cancers are harder to discover than tobacco related cancers because the symptoms are not always obvious to the individual who is developing the disease, or to professionals that are looking for it. They can be very subtle and painless. We present cases of three patients (47-year-old male, 48-year-old male and 55-year-old male) diagnosed with benign warts in the oral cavity were studied for the presence of HPV DNA. We used PCR kit for the qualitative detection of human papillomaviruses (REF V-10/14-50F) provided for us by "Sacace Biotechnologies". The targets of the PCR reaction were E6, E2, E1, L1 genes. Positive and negative controls were used to avoid false results. Our PCR analysis showed the presence of HPV- DNA within the samples.

P4 MICROBIAL DEGRADATION OF PHENOL AND PHENOL DERIVATIVES BY *ASPERGILLUS FUMIGATUS* STRAIN AL15, ISOLATED FROM ANTARCTIC SOILS

Gerginova M., Litova K., Peneva N., Alexieva Z.

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

The ability of the strain *Aspergillus fumigatus* AL15 isolated from Antarctic soils to degrade phenol and phenolic derivatives at different temperature conditions was studied. The strain could degrade and assimilate completely 0.3 g/l phenol, catechol, and *o*-cresol as a sole carbon sources at 23°C for a period of 45 h, 24 h and 26 h, respectively. At 10°C the strain was able to degrade the same quantity phenol (450 h) and catechol (144 h) but not degraded *o*-cresol for the examined period of 500 h. The intracellular enzyme activities of phenol hydroxylase (EC1.14.13.7) and catechol l,2-dioxygenase (EC1.13.11.1) were determined in cleared lyzates, derived from cells cultivated with 0.3 g/l of each one of the studied compounds included in the medium. Phenol hydroxylase activity in cells cultured at 23°C with phenol was 1.07 U/mg protein while in the experiments carried out at 10°C was 0.134 U/mg protein. Catechol l,2-dioxygenase kept relatively high values of activity in cells grown in a medium comprised phenol at 23°C (1.44 U/mg protein) and at 10 °C (1.008 U/mg protein). In the

experiments with catechol included as a sole carbon sources in the cultivation medium the activities of phenol hydroxylase was measured as 0.17 U/mg protein at both temperatures. Catechol l,2-dioxygenase activity was 4.18 U/mg protein at 23°C and 1.5 U/mg protein at 10°C. The identification and partial sequence of the genes encoding phenol hydroxylase and catechol l,2-dioxygenase in *A. fumigatus* AL15 strain was performed. The established nucleotide sequences were deposited in the NCBI under Accession numbers: KT371934.1 and KT371935.1, correspondingly.
Key words: biodegradation, phenol, *Aspergillus fumigatus*, Antarctica

P 5 LACTIC ACID MICROBIOTA OF TRADITIONAL BULGARIAN CHEESE AND YOGURT

Nemska V.[1,2], Danova S.[1]

1Department of Microbial Genetics, The Stephan Angeloff Institute of Microbiology,
2 University of Chemical Technology and Metallurgy, 8, "St. Kliment Ohridski", blvd., 1756 Sofi,

The eternal aspiration of people for health and longevity stimulates the necessity of a change in the contemporary nutrition habits and industrial practices of modern societies. This increases the interest towards the lactic acid bacteria (LAB). They play a major role in determining the organoleptic properties and healthy effects of fermented milks and related products. However, little is known on the biodiversity and beneficial role of autochthonous lactic microbiota of artisanal milk products. With this aim, several home-made samples of cheeses and yogurt are collected from Western; North-Western and Central part of Bulgaria. A rich microbiota has been estimated, together with high sanitary quality of the tested dairy products. As a result a collection of more than 100 LAB cultures was created. 72 strains, isolated from artisanal samples of cheese and yogurt, were characterized. The antimicrobial activity against *Escherichia coli* and foodspoilage bacteria was a pre-selective criterion. Selected active lactobacilli were identified according to the modern polyphasic taxonomy to the species *Lactobacillus plantarum*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus rhamnosus and Lactobacillus fermentum.* The stable domination of species from *L. plantarum and L. casei* group were shown in artisanal samples of cheese, prepared according to tradition, without industrial starters.
Key words: cheese, yogurt, lactobacilli, *Lactobacillus plantarum*

P 6 VITAMIN D DEFICIENCY AND INFECTIOUS DISEASES

Rimpova, N[1], Grozdanov P. [2], Iliev D. I.[1]

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[2]The Stephan Angeloff Institute of Microbiology, BAS

Classical understandings of vitamin D function indicate its major role in calcium and phosphorus homeostasis. The discovery that almost every cell in the human body contains the vitamin D receptor has led to the concept of its extraskeletal effects. The association of its deficiency with common cancer, autoimmune diseases, such as type I diabetes and cardiovascular diseases is of great scientific interest. A growing evidence of its immunomodulatory function and regulation of gene expression is being gathered though the past 10 years. A study among patients with the Tuberculosis infection conducted in 2006 provided the biochemical basis of its role, as vitamin D is being activated by the macrophages through a toll-like receptor, raising the production of antimicrobial peptides in the respiratory epithelia.
Although this antimicrobial mechanism of vitamin D has been demonstrated only in macrophages infected with *M. tuberculosis*, it is also well known that the defensive protein cathelicidin has broad-

spectrum activity against a wide variety of other pathogens, including gram-negative and gram-positive bacteria and fungi.
Epidemiologic studies have demonstrated strong associations between seasonal variations in vitamin D levels and the incidence of various infectious diseases, including, respiratory infections and influenza.
Worldwide, an estimated 1 billion people have inadequate levels of vitamin D and deficiency can be found in all ethnicities and age groups. This has urged leading researchers in this field to encourage further knowledge and recommend revision of the current guidelines for adequate dietary supplementation with vitamin D. The main aim of this review is to summarize current evidence on the key mechanisms of vitamin D immune system regulation and the association of its deficiency with the incidence and severity of infectious diseases in children.

P 7 BIODEGRADATION OF AROMATIC AND ALIPHATIC XENOBIOTICS BY *RHODOCOCCUS WRATISLAWIENSIS* BN38

Kabaivanova L., Christova N., Nacheva L.

The Stephan Angeloff Institute of Microbiology

The catabolic ability of the strain *Rhodococcus wratislawiensis* BN38 was followed to prove its potential for bioremediation purposes. Adaptation of the strain was carried out with multiple xenobiotic supply. After 10 passages the cells performed 22 cycles of operation realizing 11 g/l degradation of phenol compared to 3 g/l by the non adapted cells. Different concentrations of phenol and n-hexadecane were subjected to degradation by the investigated strain. The results showed that *Rhodococcus wratislawiensis* revealed capacity for simultaneous mineralization of both xenobiotics. At 500 mg/l phenol containing medium the cells showed highest biodegradation activity. During simultaneous degradation of both aromatic and aliphatic xenobiotics (in equal concentrations), 8 g/l (100%) phenol and 2.4 g/l (30%) n-hexadecane were mineralized for 16 active cycles of operation (each 18-24 h). The adapted cells of *Rhodococcus wratislawiensis* BN38 performed a successful simultaneous biodegradation of the aromatic and aliphatic xenobiotics. This capability can be further applied in different bioremediation processes.

P 8 ANTIINFLAMMATORY ACTION OF TYROSINE KINASE INHIBITORS IN MODELS OF SEPSIS AND CHRONIC ARTHRITIC DISEASES

Gyurkovska V., Ganova P., Ivanovska N.

Department of Immunology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

The janus kinase (JAK)-signal transducer and activator of transcription (STAT) cascade plays a principal role in the signaling of a vast array of cytokines and growth factors, which stimulates diverse cellular functions and immune responses. Binding of cytokines to relevant JAK receptors triggeres autophosphorylation, phosphorylation of tyrosine residues on the cytokine receptor, and phosphorylation further leading to activation of various STAT molecules. The incidence of sepsis is increasing despite the major advances in the development of antimicrobial agents and other supportive treatments. Septic patients often develop systemic inflammatory response, of which multiple organ failure is a main complication. Chronic joint inflammation arises when the acute response is not completely turned off, continuing to stimulate pro-inflammatory mediators when they may not be needed. Regulation of Jak/STAT signaling is important in controlling the immune response to sepsis. A new trend in arthritis therapy is now available due to tyrosine kinase inhibitors

possessing similar efficacy as anti-TNF therapies and moderate toxicity. This review aims to summarise data on two small molecules capable to influence tyrosine kinases (TYKs), Jak2 inhibitor tyrphostin AG490, and Jak3 inhibitor berberine. The present studies were designed to evaluate their effects in mouse models of acute inflammation (lypopolisaccharide, LPS-induced peritonitis) and chronic joint inflammation (collagenase-induced osteoarthritis, CIOA and zymosan-induced arthritis, ZIA). The inhibitors decrease the levels of critical inflammatory factors contributing to sepsis and arthritis development like IFN-γ, IL-1, IL-6 and IL-17, and diminish the generation and activation of pro-inflammatory cells, like F4/80 macrophages and of RANKL positive cells.
Key words: arthritis, berberine, kinase inhibitors, sepsis, tyrphostin AG490

P 9 DETECTION OF *YERSINIA ENTEROCOLITICA* IN PIG FAECES AND TONSILS BY LAMP

Zaharieva M.[1], Gatzovska M.[1], Teneva V.[1], van Collie E.[2], Heyndricks M.[2], Najdenski H.[1]

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[2]*Unit "Food Safety", Institute for Agriculture and Fisheries Research (ILVO), Brusselsesteenweg 370, Melle, Belgium*

Yersiniosis is the third most commonly reported zoonosis in the European Union. *Yersinia enterocolitica* is the most often isolated causative agent from humans, but natural reservoir of this enteric pathogen are the pigs. Significant difficulties for its fast detection represent the time consuming and laborious bacteriological methods, as well as the low sensitivity of the conventional end-point PCR analysis. The aim of the current study was to establish fast, specific, sensitive and inexpensive LAMP based methodology for detection of the pathogen in pig faeces and tonsils.
In total, 75 samples, isolated from pig faeces were examined for the presence of *Y. enterocolitica* through the ISO standard methods and parallel direct DNA isolation, followed by LAMP identification. The used LAMP primer set was complementary to the gene *phoP*. Its selectivity was tested on 16 *Y. enterocolitica* strains (including 6 pathogenic strains of four serotypes: O:3, O:5, O:8, O:9) and 16 other Gram (-) and Gram (+) bacterial species. The sensitivity of the method was determined through artificial contamination in comparison with TaqMan-qPCR. Classical CFU detection on agar plates served as a control. The DNA isolation protocol was established by screening of several commercially available kits. The visualisation of the results was performed with hydroxynaphthol blue and DNA electrophoresis in agarose gel.
The selected primer set was strongly selective for pathogenic *Y. enterocolitica* strains. The sensitivity of the LAMP assay performed with pure DNA input was 1 to 10 DNA copies per reaction. The sensitivity in contaminated samples corresponded to that of the TaqMan-qPCR, but was limited by the DNA isolation method. The direct dye visualisation of the samples was comparable to the gel electrophoresis result. In conclusion, the LAMP method is reliable, fast, selective and sensitive but further optimisation of the DNA isolation protocol is needed in order to enhance its sensitivity.

P 10 DEVELOPMENT OF PLANT *IN VITRO* SYSTEMS FROM THE GENUS *SALVIA* TOWARDS BIOSYNTHESIS OF BIOLOGICALLY ACTIVE TRITERPENES

Marchev A., Georgiev V., Pavlov A.

Laboratory of Applied Biotechnologies – Plovdiv, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 139 Ruski Blvd., 4000 Plovdiv, Bulgaria

Plants from the genus *Salvia* are abundant natural source of triterpenes. Triterpenes exhibit numerous valuable biological activities, *e.g.* anti-inflammatory, cytotoxic, antivirus and antimicrobial, which makes them attractive for pharmaceutical and cosmetics industry. The latest scientific discoveries reveal the potent hepatoprotective, antitumor, anti-inflammatory and antireumatic activities of ursolic and oleanolic acid, which provokes the interest of many pharmaceutical companies to incorporate them in many drugs. As a result of the growing market demand raises the necessity from the development of alternative technologies for triterpenes production. The induction and utilization of plant cell suspension cultures and hairy roots from species of the genus *Salvia* might be an appropriate platform for their sustainable biosynthesis. Currently we summarize the latest investigations from a working group in the Laboratory of Applied Biotechnologies in Plovdiv, which is focused on induction and assessment of the biosynthetic potential of diverse plant *in vitro* systems from rare Bulgarian *Salvia* species and establishment of biotechnological process for biosynthesis of biologically active triterpenes.

Key words: *Salvia*, triterpenes, plant *in vitro* systems

P 11 STUDY OF BACTERIOCINS FROM LACTIC ACID BACTERIA

Stoyancheva G.[1], Stoyanov A.[1], Miteva-Staleva J.[2], Kostadinova N.[2], Krumova E.[2]

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Lactic Acid Bacteria bacteriocins are a very heterogeneous group of compounds. They are small peptides, which are active through the formation of pores and efflux of small metabolites from sensitive cells or through enzyme inhibition. The gene-encoded nature of bacteriocins makes them easily amenable through bioengineering to either increase their activity or specify target microorganism and higher stability. The bacteriocins have a number of positive attributes that have made them especially attractive for various applications: in food industry, in pharmaceutical industry, in veterinary industry.

In our studies we identify LAB strains from different Bulgarian ecosystems, potentially bacteriocin producers, determine their antimicrobial activities, analyze the genes encoding bacteriocins and determine primary the physicochemical nature of those active substances.

Key words: bacteriocins, *Lactobacillus*, genes

P 12 ROLE OF MIGRATORY BIRDS ON DISSEMINATION OF SOME BACTERIAL ZOONOTIC AGENTS

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Avian mobility and migration are crucial epizootological factors for the spread of several zoonoses. Their causative agents can be transmitted between species at breeding, wintering and stop-over places. Migratory birds may act as conveyers and could be involved in carriage of microbial

pathogens by three mechanisms: 1) as biological carriers; 2) as mechanical carriers e.g. through their excreta (cloacal mixture of feces and urine); and 3) as hosts and carriers (transporters) by transporting infected hematophagous ectoparasites as vectors of diseases through long distances. In tick-borne infections, larval or nymphal ticks are known to remain attached to the host and then to drop off in a new geographic area during bird migration. In food- and waterborne infections, the agent can be shed by infected migratory birds, resulting in contamination of soil and environment. The available data about bird species, seasonality, geographic regions of occurrence and European migratory flyways are presented and discussed with a focus on migratory birds as hosts and carriers of the most frequent zoonotic bacterial pathogens - campylobacteriosis, salmonellosis, yersiniosis, Lyme borreliosis, etc. A better understanding of avian migration patterns and birds' potential role in the geographic expansion of infectious diseases would help their prediction and prevention of future outbreaks of infections among other birds, farm and wild animals, and humans.
Sufficient knowledge on this spectacular phenomenon could elucidate the complex interrelationships that exist between the emerging infection diseases of humans, animals and wildlife, e.g. the impact of wild birds on transmission of drug-resistant bacterial species.
Key words: migratory birds, zoonoses, drug resistance

P 13 UTILIZATION OF MIXED STARCH-INULIN SUBSTRATES BY *LACTOBACILLUS PARACASEI*

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Being inhabitants of a fermented cereal beverage, *L. paracasei* strains B41 and LC1, possess amylase in addition to inulinase activity. To examine the genetic control on *amy1* and *inu* genes in media with mixed polysaccharide content, RT-PCR with specific primer pairs were carried out. Total RNA from *L. paracasei* strains B41 and LC1 cultivated in MRS (20 g L^{-1} glucose), starch, inulin, or mix of the two substrates in different proportions (1/3 to 3/1) were isolated. The obtained copy-DNA was used as a template for PCR with the described primer pairs. The results revealed that *amy1* and *inu* genes were expressed in the cells, grown in all media containing starch, inulin, or mix of them, gaining strong positive bands. Both genes transcription was entirely repressed by glucose. These conclusions were proved over again by Northern dot hybridization of the described above total RNAs with *amy1* and *inuB41* dig-labeled probes. The simultaneous utilization of the mixed polysaccharides starch and inulin by *L. paracasei* B41 was studied during batch processes without pH control in model media, containing soluble potato starch and Frutafit®HD in different proportions. The biomass growth after 48 h of fermentation showed that inulin was the substrate gaining greater biomass accumulation. However, the amounts of LA, produced from starch, inulin and mixed substrates were similar, and the starch was fully consumed, leading to the conviction that the strain was capable to utilize both substrates to an equal extent.

P 14 DRUG TOLERANCE TO ETHAMBUTOL OF *MYCOBACTERIA* CELL WALL DEFICIENT L-FORMS

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The loss of cell wall in mycobacteria results in appearance of highly pleomorphic forms with new biologic characteristics, known as L-forms. It is known that L-forms occur along with resistance to factors that trigger their appearance and survive unfavorable conditions much longer than the classic

bacteria. In this respect, it was of interest to study the relationship between L-form formation in *M. tuberculosis* and exhibition of drug tolerance to Ehambutol (EMB), which acts through the inhibition of cell wall synthesis.

Model using *in vitro* cryogenic stress treatment and subsequent cultivation in two variants of Middlebrook 7H9 semisolid medium (EMB containing medium in concentration of 2mg/l and control medium) was used for induction and selective isolation of L-forms both from EMB sensitive (No18) and resistant (No 43) strains of *M. tuberculosis*. The obtained L-form variants of tested strains gave rise to colonies with typical "fried eggs" shape. Electron microscopy showed specific morphology and ultrastructure of cell wall deficient bacterial population. EMB resistance/susceptibility was evaluated phenotypically and by PCR assay targeting *embB 306* mutations. It was found that L-forms originating from a sensitive No18 strain and obtained after cultivation in both variants of semisolid medium (with and without EMB) showed phenotypically tolerance to high concentration of EMB (16mg/l). It is interesting to note that amplification of *embB 306* failed in all L-form variants of both *M. tuberculosis* strains, although all L-forms were confirmed by IS6110 Real Time PCR.

We suggest that loss of specific targets for EMB in L-forms of M. tuberculosis may underlie the phenotypic drug tolerance to this drug.

Key words: *M. tuberculosis*, ehambutol, L-forms, drug tolerance

Wednesday, March 15th, 2017

Scientific session II

Plenary lectures

9.00 - 9.30 OSMOLYTES FROM MESOPHILES TO HYPERTHERMOPHILES AND BACK

PL 5 Milton S. da Costa

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Many organisms that live at very high temperatures have been isolated from shallow marine and abyssal thermal environments, where the geothermal water may reach the salinity of the surrounding seawater. These organisms, like all other microorganisms must adjust, within intrinsic limits, to alterations in the water activity of the environments. The majority of microorganisms adjust osmotically by the selective accumulation of small molecular weight organic compounds. Thermophiles and hyperthermophiles accumulate a few compatible solutes that are also common in mesophilic bacteria and archaea, namely trehalose and glutamate and even the very rare glucosylglycerate (GG). However, the majority of the compatible solutes encountered in hyper/thermophiles are unique to these organisms. These compatible solutes include mannosylglycerate, di-myo-inositol-phosphate and the very rare compatible solutes di-glycerol-phosphate, di-mannosyl-di-myo-inositol-phosphate and mannosylglyceramide.
In recent years we have studied the synthesis of mannosylglycerate (MG) and trehalose in *Thermus thermophilus* (Phylum *Deinococcus*/*Thermus*), *Rubrobacter xylanophilus* (Phylum *Actinobacteria*) and *Persephonella marina* (Order *Aquificales*), among other thermophilic and mesophilic organisms. Unlike other species of this genus, the species *Thermus thermophilus* is capable of growing in media containing 3 to 5% NaCl. The strains of *T. thermophilus* accumulate primarily trehalose and lower levels of mannosylglycerate (MG) during osmotic adjustment. Recombinant mutants lacking the genes for the synthesis of trehalose, MG or both, result in a profound effect on the ability of organisms to grow in media containing NaCl. These studies led us to encounter a gene in the species of *Mycobacterium* that leads to the synthesis of GG that is bound to a polysaccharide but does not serve as a compatible solute. Other compatible solutes have been identified in some deep-branching species of bacteria will be discussed, namely mannosylglycosylglycerate (MGG) and the recent discovery of genes and their enzymes for the degradation of these compatible solutes will also be presented.

9:30 – 10:00 HARNESSING BENEFIT FROM TARGETING TUMOR
PL 6 ASSOCIATED CARBOHYDRATE ANTIGENS

Tomas Kieber-Emmons

University of Arkansas, USA

Integrating additive or synergistic antitumor effects that focus on distinct elements of tumor biology are the most rational of strategies for cancer treatment. The real challenge is to define what elements of tumor biology make the most sense to be targeted. Signal transduction (pathways) can define therapeutic strategies and approaches that might be tailored to harness benefit from sustained immunity much like that observed from natural antibodies involved in immune surveillance mechanisms. Tumor Associated Carbohydrate Antigens (TACAs) are pan-targets on tumor cells because they play roles in initiation and metastases of cancer, and considered as common targets shared by many tumor types, and regulating a network of signaling pathways associated with cell survival. Strategies that target TACAs therefore have potential benefit as cell death therapies. We

have been developing an active vaccination strategy targeting TACAs using carbohydrate mimetic peptides (CMP) designed as pan-immunogens. One CMP called P10s was computer designed to induce anti-GD2 and anti-LeY antibodies with the intent of inducing multiple sets of antibodies reactive with multiple TACAs when immunizing with a single agent. Our CMP is now being tested in Phase II clinical trials in breast and lung cancer, showing that P10s can induce proapoptotic antibodies.

10:00 – 10:30 NEOPTERIN PRODUCTION AND TRYPTOPHAN BREAKDOWN
PL 7 IN INFECTIOUS DISEASES

Dietmar Fuchs

Division of Biological Chemistry, Biocenter, Medical University, Innsbruck, Austria

Cellular (Th1-type) immune response is a crucial aspect within the host's response against infections with viruses and other intracellular pathogens. It is mainly operated by cytokines interleukin-2 (IL-2) and interferon-γ (IFN-γ), whereby the latter triggers a broad range of antimicrobial activities most of them representing anti-proliferative aspects. Among them the activation of enzymes like indoleamine 2,3-dioxygenase (IDO-1), inducible nitric oxide synthase (iNOS) and GTP-cyclohydrolase I (GTP-CH-I) are key elements. GTP-CH-I is the central enzyme in the production of neopterin and of iNOS cofactor tetrahydrobiopterin (BH_4). IDO-1 metabolizes tryptophan and as a consequence deprives this essential amino acid of protein biosynthesis, and iNOS strongly impacts on iron metabolism. Aside from its prominent role in antimicrobial and antitumoral host defense, IDO-1 is involved in the activation of regulatory T-cells and thus in immunoregulation. The monitoring of the biomarkers neopterin production and tryptophan breakdown in infectious diseases can be applied to predict outcome and treatment efficacy. The additional measurement of neopterin concentrations is mandatory in Austria for blood donor screening to reduce the risk of virus transmission via blood transfusion.Biochemically, tryptophan is not only component of proteins but also precursor molecule of the neurotransmitter serotonin and of the neuroactive kynurenine derivatives like the neuroprotectant kynurenic acid and its counterpart, the neurotoxin quinolinic acid. On the one hand, immune response may results in serotonin deficiency and, on the other hand, kynurenic and quinolinic acids can accumulate when IDO-1 is activated. The metabolic disturbances could relate to some neuropsychiatric deviations, which are often observed in patients suffering from infections especially in the acute phase and when becoming chronic.

11:00 – 11:30 NEW FACETS THE MECHANISM OF ACTION OF THE
PL 8 ALKYLPHOSPHOCHOLINE IN ERUFOSINE

Martin Berger

Toxicology and Chemotherapy Unit, German Cancer Research Center, Heidelberg, Germany

The ether lipid analogue erufosine (erucylphospho-N,N,N,-trimethyl-propylammonium) is an antineoplastic agent classified as a third generation alkylphosphocholine (APC). The mode of action of APCs involves disturbance of phospholipid metabolism and of membrane lipid raftmediated signaling. APCs have shown significant cytotoxic and pro-apoptotic activities towards a number of tumors cell lines by modulating members of the JNK 1/2, Raf/MEK/ERK, and PI3K/Akt/mTOR signaling pathways and inhibition of cell division. Recent microarray findings revealed that erufosine caused induction of RhoB, however, combination of erufosine with $siRNA^{RhoB}$ knockdown disproved RhoB's involvement in its mechanism of action. Another finding was that cyclins and CDKs were down regulated in a dose dependent manner. These results were verified at both, mRNA and protein levels. Erufosine not only caused G2M block but also caused inhibition of colony formation thus preparing the oral squamous carcinoma cells (OSCC) to undergo apoptosis. We are the first to show that erufosine caused inhibition of tumor growth in an OSCC xenograft model and caused down

regulation of cyclin D1, CDK4 and CDK6. These findings collectively show the potential of erufosine to be used as a cell cycle inhibitor in OSCC progression and support its further evaluation in cancer treatment, alone, or in combination.

11:30 – 11:50 "ONE HEALTH" APPROACH TOWARD ANTIMICROBIAL RESISTANCE
PL 9 Gabriel Ionescu

"Carol Davila" University of Medicine and Pharmacy, "Cantacuzino" National Institute of Research, Bucharest, Romania

Health care was marked in the last decades by some initiatives at global, regional and national level, which had a huge impact on the improvement of the quality of life. But these achievements are threatened by a new danger: antimicrobial resistance.

Worrying recent information coming from professionals in epidemiology, microbiology and infectious diseases was taken by officials and opinion leaders around the world that drew attention to the phenomenon of antimicrobial resistance as a big challenge for health care systems worldwide. As Dr. Keiji Fukuda - Assistant Director-General of the World Health Organization - said, "By 2050 more people will die from antibiotic-resistant infections than currently die from cancer". The lack on the market of new antibiotics against bacteria resistant to the existing ones has raised the risk of the rapid spread of pan-resistance and the return to the pre-antibiotic era. One of the most recent proposal for this public health emergency is the "One Health". This is a holistic approach of global problems and seems to become a new hope for "unsolved issues". "One Health" is an integrative effort of multiple disciplines to reach the health for people, animals, and the environment. *One Health Day* was celebrated on the 3rd of November 2016 with 156 One Health events held in 37 countries and *World Antibiotic Awareness Week* was held from the 14th- 20th of November 2016. One day, one week or one month to fight against antimicrobial resistance are good initiatives for raising awareness, but it is only a starting point, and it is too little. The world needs to act urgently.

11.50 – 12.10 CONTROL OF ENTEROVIRUS CIRCULATION IN THE POST-CERTIFICATION
PL 10 PERIOD OF POLIO ERADICATION

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Institut Pasteur, Saint-Petersburg, Russia

The complex enterovirus surveillance can guarantee effective control of enterovirus circulation among the population in order to maintain the polio free status of the country in the post-certification period. As wild poliovirus type 1 - the causative agent of the outbreak of poliomyelitis in Tajikistan in 2010 was isolated from some migrants in the Russia we compared enteroviruses isolated from migrants to viruses that circulate in Russia. The predominant enteroviruses isolated from the patients with enterovirus infection were Coxsackieviruses A, Coxsackieviruses B1-6, Echovirus 6 and Echovirus 30. Outbreaks of hand, foot and mouth disease were registered in the North-West of Russia in 2011-2012 and Coxsackievirus A16 which had not previously been detected there was isolated from patients. The identification of two genetic variants closely related to strains isolated in France in 2010 and in Japan in 2011 suggested that Coxsackieviruses A16 which provoked outbreaks had been brought to the North-West of Russia through two importation events. The enterovirus Echo30 lineage which largely circulated in Russia in 2013 and caused meningitis outbreaks belonged to the new genotype H. Viruses that caused outbreaks were closely related to the strains of genotype H detected in China in 2010-2013. Since Echo30 previously isolated in Russia belonged to genotype Ec2, genotype H which had not been detected in the country earlier is likely to have been imported into Russia from South-East Asia. Coxsackieviruses B1-6 isolated from healthy resident children had a different origin according to phylogenic analysis. Coxsackieviruses A13, 17, 24, enteroviruses 99 and 75, which had not circulated earlier in the North-West of Russia, were isolated from migrants' children who arrived from unsafe territories.Coxsackievirus A13 was isolated from a Tajik child infected by the wild poliovirus type 1. The enterovirus surveillance

gives us new information about enterovirus circulation among various groups of population, which is indispensable for prevention of enterovirus infection and for limitation of circulation of imported new serotypes/genotypes of enteroviruses by means of systematic examination of groups at risk.

12.10 – 12.30 PASTEUR INSTITUTE IN NOVI SAD, SERBIA, 95 YEARS ON RABIES
PL 11 PROPHYLAXIS

Dušan Lalošević, *Pasteur Institute in Novi Sad, Novi Sad, Serbia*

After more than 95 years of Pasteur Institute in Novi Sad works on the prophylaxis of rabies, this scariest infectious disease slowly goes down in history. Today, the number of rabid animals in Serbia is so small, thanks to the European project of vaccination of foxes, that looks as it will never be. However, decades earlier, when the number of rabid domestic and wildlife animals was a big ("as in medieval epizootics of rabies", A. Hempt 1929), Pasteur Institute in Novi Sad as a central institution for the prophylaxis of rabies in Serbia, worked very successfully that these disease in humans becomes extremely rare in the former Yugoslavia and for over 35 years is gone, although until a few years were a lot of injured patients from proven rabid animals. Founder of the Institute, Dr. Adolf Hempt introduced the first in the world, completely dead rabies vaccine from nervous tissue at 1925. He's vaccine was in usage for decades in many European countries. Cell culture rabies vaccine we introduced in nineties. Twenty-five years of partnership between The National Blood Transfusion Institute of Serbia, Belgrade, and the Pasteur Institute, Novi Sad, is based on the mutual goal of producing human rabies immunoglobulin (HRIG). Pasteur Institute in Novi Sad today is the national referent institution for rabies in Serbia, certified by European Union Reference Laboratory (ANSES, Nancy) for rabies diagnostics and serology.

Scientific session III

Plenary lecture

14.00 – 14.30 EPIDEMIC RESISTANT GENOTYPES OF *MYCOBACTERIUM TUBERCULOSIS*
PL 12 EMERGING IN RUSSIA AND FORMER SOVIET UNION COUNTRIES

Igor Mokrousov, *St. Petersburg Pasteur Institute, St. Petersburg, Russia*

Mycobacterium tuberculosis has clonal population structure whereas its different lineages are marked with different evolutionary pathways, and some of them have undergone a dramatic global dissemination. Furthermore, clinically/epidemiologically significant compact clusters have been identified by high-resolution genotyping. I will review some *M. tuberculosis* clones from different genotype families (Beijing, Latin-American Mediterranean, NEW-1/Iran) that emerge in Russia and other countries of the Former Soviet Union and have impact on health situation in EU.
For example, a strong association with multi/extensively drug-resistant TB has been shown for Beijing B0/W148 strain that I termed a successful Russian clone. It likely originated in Siberia and its primary dispersal was driven by a massive population outflow from Siberia to European Russia in the 1960-80s. Its successful dissemination was triggered by an advent and wide use of the modern anti-TB drugs and was due to its remarkable capacity to acquire drug resistance. For another significant genetic family of *M. tuberculosis*, LAM (Latin American Mediterranean), we also found an emerging epidemic sublineage that is actively spread within the European part of Russia and Eastern Europe. Mass immigration and a high prevalence in the initial population represent decisive factors that define the spread of M. tuberculosis strains. On the other hand, manifestation of the strains's pathobiology can be changed (counteracted) by genetic factors of the human host population. Until very recently, Beijing genotype isolates were not detected in autochthonous populations in Eastern Europe despite the close links with Russia/USSR in the recent and historical past (Bulgaria being the most impressive example). Speculatively, a kind of human resistance is developed in local population through its co-existence with historical local clones, and acting against imported clones.

Oral presentations

14:30 – 14:40
OP 1

S-OXYGENATION OF DITHIOCARBONIC ACID DERIVATIVES (XANTHATES) BY FLAVOPROTEIN MONOOXYGENASES AS THE PRIMARY MECHANISM OF THEIR ANTIMYCOBACTERIAL ACTIVITY

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[1]*Institute of Neurobiology, Bulgarian Academy of Sciences*
[2]*Institute of Microbiology, Bulgarian Academy of Sciences*

Global tuberculosis epidemic has been complicated by the recent worldwide emergence of multidrug-resistant strains of *Mycobacterium tuberculosis*. Recent attempts to potentiate the existing antimycobacterial activities, such as ethionamide (ETH) and other drugs, have increased the possibility for targeting resistance. ETH plays a central role in the treatment of patients that exhibit resistance to the front-line drugs. The ETH, thioamide and thiourea class of antituberculosis agents encompasses prodrugs that are oxidatively converted to their active forms (S-oxides) by the flavin monooxygenase EtaA of *M. tuberculosis.* The activated forms react with nicotinamide adenine dinucleotide (NAD+) to form an ETH-NAD adduct. This adduct inhibited the common target InhA, the NAD-dependent enoyl-ACP reductase of the fatty acid synthase type II system, resulting in the inhibition of mycolic acid biosynthesis and cell lysis.
Here we would like to present our results with different derivatives of dithiocarbonic acid (xanthates) after oxidative transformation with purified EtaA and the possibility this reaction to be in the basis of their antimycobacterial activity. With an NADPH regenerating system and purified EtaA, different xanthates were oxidized to single product – corresponding S-oxides (sulfines or perxanthates), products similar to ETH oxidation. The enzyme activity was not inhibited in the time of reaction. Higher transformation activity was shown towards xanthates derivatives with longer alkyl chain. Thus the kinetic constants for octyl-xanthate EtA oxidation to perxanthate are similar to those obtained for ETH as substrate (Km=5 μM; Vmax = 1.023 nmolP/min; kcat = 5.2 molP/min/molE and 194 μM; 1.46 nmolP/min; 7.73 nmolP/min, correspondingly. Our first results for testing the antimycobacterial activity of two xanthates derivatives, determined *in vitro* against *M. tuberculosis* H37Hv using REMA colorimetric assay, showed high antituberculosis potency with MIC below 20 μg/ml, compare with that of ETH. For the antimycobacterial activity of xanthates could be consider some other effects on cell signaling system like inhibition of phospholipase C and thioredoxin reductase activities leading to cell apoptosis.

14.40 – 14.50
OP 2

FROM LABORATORY TO THE PLANT. SPECIFIC REQUIREMENTS AND ATTEMPT FOR EFFICIENT INDUSTRIAL APPLICATION OF FERMENTATION TECHNOLOGIES

Ratkov A., Dimov I., Kristeva J., Filipov F., Petkova R.

The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

Objectives: The processes for microbial production of amino acids are among the most important in terms of tonnage and economical value. Permanent growing market for amino acids led to significant improvements in bioprocess and downstream technology as well as in molecular biology. During the last two decades big efforts were made to increase the productivity and to decrease the production cost.
Methods: Classical breeding methods and mutagenesis have been applied to channel the metabolic partway in the biosynthesis of different amino acids followed by screening for mutants with different

characteristics. Comparative studies of efficiency of the applied different methods for cultivation of the chosen producers have been done.
Results and Conclusions: The presentation gives a short overview of the world market for amino acids. Attempt and achievements in investigation and developments of the technologies for some amino acid production at the Institute of Microbiology, Bulgarian Academy of Sciences are summarized. Developments and achievements in bioprocess technology i.e. development of lab-scale, semi-industrial and production scale technology as well as specific requirements and attempt for efficient industrial application of the technologies are shortly discussed.
Keywords: amino acids, repeated fed-batch cultivation, industrial applications

14.50– 15.00 INFLUENCE OF LOW TEMPERATURE ON THE DEGRADATION
OP 3 ABILITY OF FUNGAL STRAINS ISOLATED FROM ANTARCTIC SOILS

Gerginova M., Litova K., Peneva N., Alexieva Z.

Department of General Microbiology, Section of Microbial Genetics, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

The ability of the isolated from Antarctic soils mold strains to degrade phenol, phenolic derivatives and polyaromatics at different temperature conditions, respectively at 10 °C and 25°C was established. The aromatic compounds were added to the mineral nutrient media for cultivation as sole carbon sources. The results have shown the strains *Penicillium commune* AL5 and *Lecanicillium* sp. AL12 degraded phenol and its hydroxylated and methylated mono-derivatives at both temperature regimes, which indicated its psychrotolerant character.
The experiments carried out with PAHs (naphthalene, anthracene and phenanthrene) demonstrated the capability of *Penicillium commune AL5* to grow and utilize 0.3 g/l of each examined compound as a sole carbon source at both examined temperatures for cultivation.
The strain *Lecanicillium sp.* AL12 degraded 0.3 g/l naphthalene and anthracene but not degraded 0.3 g/l phenanthrene at the same experimental conditions.
The data demonstrated specific capabilities of the studied strains to degrade aromatic compounds (phenol, catechol, resorcinol, hydroquinone, cresols, naphthalene anthracene and phenanthrene) included as a sole carbon source in the nutrient culture media. The values of enzyme activities of the first two enzymes, respectively phenol hydroxylase (EC1.14.13.7) and catechol 1,2-dioxygenase (EC1.13.11.1) involved in the ortho- pathway of phenol biodegradation in the investigated strains' cells were determined. The both enzymes showed broad substrate specificity.
The studied Antarctic fungal strains may be determined as psychrotrophic, toxitolerant microorganisms which may grow and develop in nutrient limited conditions (oligotrophs). As a conclusion we can consider that the strains studied by us may be defined rather as real polyextremophiles.
Key words: biodegradation, phenol, PAH, mold, Antarctica

15.00 – 15.10 BENEFICIAL MICROBIOTA OF TRADITIONAL BULGARIAN
OP4 DAIRY PRODUCT

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Fermented milk products possess various nutritional and therapeutic properties. They are widely accepted as functional foods, with long history of safety use. Lactic acid bacteria play (LAB) a major role in determining the organoleptic properties and healthy effects of fermented milks and related products. However, little is known on the beneficial role of autohtounous lactic microbiota of artisanal milk products,. With this aim, intensive studies on a group of 78 Bulgarian lactic acid bacteria (LAB), isolated from artisanal samples of cheese, yogurt and katak, were carried out. Two major axes were followed: food biopreservation and possible beneficial effects on the consumers' health. The antimicrobial activity against *Escherichia coli, Bacillus subtilis, Candida albicans* and *Aspergillus* sp. was a pre-selective criterion. In addition, estimation of functionality and technological relevance of active LAB were applied. As a result a group of 10 original strains with probiotic potential was selected. They were identified according to the modern polyphasic taxonomy. *Lactobacillus plantarum* and *Lactobacillus bulgricus* strains possess a broad spectrum of activity and high transit tolerance in gut conditions. The host could benefit from their ability to compete the *E. coli*, preventing biofilm formation and anti-oxidant activity. They were estimated as bio-preservative/probiotic adjuncts to traditional Bulgarian fermented products with a favorable influence on their organoleptic properties and shelf-life. The obtained results about the active *Lactobacillus* strains seemed promising for the development of new approaches for design of functional foods and to tackle a variety of new specific consumers' demands.

Key words: Cheese, Yogurt, katak, lactobacilli, Probiotics**Key words:** cheese, yogurt, lactobacilli, *Lactobacillus plantarum*

15.10 – 15.20 POSTTRANSCRIPTIONAL GENE SILENCING AS A TOOL TO
OP 5 CONTROL COXSACKIEVIRUS INFECTIONS

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The posttranscriptional gene silencing is one of the tools used to control viral replication in epigenetic level. In our experiments we use polymerase complex of bacteriophage Phi6 to produce dsRNAs, complimentary to preselected conserved gene region from the viral genome. This dsRNAs we cut to a pool of siRNAs, which induce posttranscriptional gene silencing of the chosen region and thus block the viral replication of Coxsackieviruses in vitro in Hep 2 cell monolayer. The used concentration have no cytotoxicity and block the viral replication of CVB to almost 100 % with a Selectivity Index ranging from 430 to 770.

Key words: PTGS, CVB, Hep 2 cells

Scientific session IV

Oral presentations

15.40 – 15.50 ANAEROBIC BIODEGRADATION OF LIGNOCELLULOSIC WASTES
OP 6 FOR BIOHYDROGEN AND BIOMETHANE PRODUCTION-STUDY AND MATHEMATICAL MODELING

Hubenov V., Kabaivanova L., Denchev D., Mihaylova S., Lakov V., Chorukova E., Simeonov I.

Department of Applied Microbiology, Research group "Mathematical Modeling and Computer Methods", The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

The aim of the paper is to present some aspects of the anaerobic digestion process with biohydrogen or biomethane production using lignocellulosic wastes and anaerobic microbial consortia. The opportunities for realization of a two-phase biotechnological process as well as some advantages in comparison with the one stage methane production were discussed. The results from this study reveal that the use of cells separated as inoculums in concentration of 100 mg dry cells/ g substrate stabilize the process and it takes place without given methane release. The hydrogen concentration in the biogas reached 20-40 %. The maximum yield of hydrogen and the degree of biodegradation is obtained by alkali-treated straw. Our results confirmed that the two-phase process allows the energy yield to be increased by 34,3% compared to the single-phase process. Mathematical modeling of the two-phase anaerobic biodegradation with production of biohydrogen and biomethane is also discussed. The assessment of the quality of the process using extremum seeking control as a type of optimum control strategy is used in situations where mathematical model of the process is unknown or containing uncertain parameters.
Key words: anaerobic digestion, biohydrogen, biomethane, mathematical modeling

15.50 – 16.00 GLOBAL ANALYSIS OF CO_2 FIXATION SYSTEM FROM FLUE GAS IN
OP 7 PHOTOBIOREACTORS (PBRS) BY USING MICROALGAE

Kroumov A.[1], Scheufele F.[2], Trigueros D.E.[2], Espinoza-Quiñones F.R.[2], Borba C.[2], Hinterholz C.[2], Módenes A.[2]

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Global warming connected with green energy production are the hottest topics for industry, technology, and life science in scientific projects all over the World. Special attention on this topic is given in HORIZON2020 European program. Hence, microalgae cultures and their potential for industrial application in CO_2 fixation from flue gases for second/third generation biofuels and many other low, medium and high value metabolites has received much attention in recent years. This work combines all the key knowledge about the chemical, biochemical and physical phenomena-taking place in photobioreactor (PBR), where CO_2 is fixed by using microalgae. The achievements in the field of modeling of PBRs are systematized according to system analysis principles. Hence, a global analysis of CO_2 sequestration by algae in closed PBR has been performed. On the bases of a specific application of this theory we were able to develop a complex PBR phenomenological model, which showed the power of such approach to describe sophisticated systems. Simulation example with the developed complex phenomenological model of PBR performance showed some new findings when experimental data on different sub-system were not sufficiently comprehensive.

Keywords: system analysis, CO_2 mitigation, flue gas, modeling, algal kinetics

16.00 – 16.10 ANTIMICROBIAL AND CYTOTOXIC POTENTIAL OF EXTRACTS AND
OP 8 COMPOUNDS DERIVED FROM THE PLANT G*EUM URBANUM* L.

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The increasing resistance of pathogenic bacteria to antibiotics is a global health problem. *Geum urbanum* L. posses wide range of biological activities according to ethnopharmacological data. Aim of our study was to investigate the phenolic content, antimicrobial, anti-quorum sensing and cytotoxic potential of extracts and compounds from *Geum urbanum.*
Roots and stems were used to obtain petroleum ether, ethyl acetate, *n*-butanol, water and 20% ethanolic extracts. Isolated nine single compounds were characterized by nuclear magnetic resonance. Minimal inhibitory and bactericidal concentrations (MIC/MBC) were estimated by broth microdilution method and by measuring the activity of dehydrogenase. Antibacterial rate was determined by time kill assay. Swarming motility and synthesis of pyocyanin were investigated on *P. aeruginosa* (PA01). The cytotoxicity was tested on normal and tumor cell lines by MTT assay. IC_{50} values were calculated with GraphPadPrizm.
All extracts exhibited antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus cereus*. The ethyl acetate extracts showed highest activity due to the phenol content. The IC_{50} on cell lines corresponded to MIC and MBC depending on the extract type. Both, ethyl acetate and *n*-butanol extracts inhibited the swarming motility and synthesis of pyocyanin of PA01. Tormentic acid possessed the highest biological activity among all compounds.
Geum urbanum L. is perspective new source of bioactive compounds. Antibacterial features of investigated extracts reveal their possible application for treatment of skin infections. The observed anti-swarming activity could inhibit biofilm formation. The established corresponding cytotoxicity supposes activity against bladder cancer with favorable antibacterial effect.

Poster session II

16.10 – 16.50 Poster presentation and discussion

P 15 EFFECTIVE COMBINATION OF S-ADENOSYL-L-METHIONINE AND OSELTAMIVIR ON EXPERIMENTAL INFLUENZA VIRUS INFECTION IN MICE

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Depletion of endogenous low-molecular antioxidants, and especially of glutathione, in influenza virus infection causes severe pathology and complications, i.g. changes in the intracellular redox balance, increased production of reactive oxygen species, development of oxidative stress, and vascular hyperpermiability. S-Adenosyl-L-methionine (SAM) has been shown to reduce lung pathology in inflammatory conditions.

The present study was designed to investigate the effect of the combination of SAM as a precursor of glutathione, and the specific neuraminidase inhibitor of influenza type A virus oseltamivir in experimentally infected mice. Infected animals were supplemented with SAM in a single daily dose of 100 mg/kg, starting from 5 days before infection until day 4th after viral inoculation. Oseltamivir was applied in a daily dose of 2.5 mg/kg in two intakes for 5 days, starting from 4th hour before infection. End-point evaluation was 14 day survival rate, average survival time, index of protection, and viral load in lungs.
The results showed that application of SAM alone did not show significant antiviral prevention. In mice supplemented with oseltamivir only in a daily dose of 2.5 mg/kg, survival rate was 70%, the viral load was 4. Combination therapy of influenza infected mice with oseltamivir and SAM led to rising of 90% of protection and lowering the viral load with abouth 30%. The present findings suggest that combined therapy of SAM as a precursor of glutathione and the specific inhibitor of influenza virus replication oseltamivir could be effective on modulation of host defense mechanism(s) at doses that are three times lower than the therapeutic.
Key words: influenza infection, S-adenosyl-L-methionine, oseltamivir
Acknowledgments: The authors express their gratitude to Program for career development of young scientists, BAS, 2016.

P 16 *RAINEYA TEPIDIPHILA* GEN. NOV., SP. NOV. A SLIGHTLY THERMOPHILIC BACTERIUM OF THE PHYLUM 'BACTEROIDETES' AND THE PROPOSAL OF *RAINEYACEAE* FAM. NOV.

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One isolate designated, SPSPC-11^T, with an optimum growth temperature of about 45-50ºC and an optimum pH for growth between 7.5 and 8.0, was recovered from a reddish biofilm at the hot spring at São Pedro do Sul in Central Portugal (40º 46' N, 8º 4' W). Based on 16S rRNA gene sequence and phylogenetic analysis the new organism is most closely related to the species of the genus *Thermonema* with 16S rRNA gene pairwise sequence similarity of 82 to 83%. The isolate was orange pigmented and formed non-motile rod-shaped cells that stain Gram-negative. The organism was strictly aerobic, oxidase and catalase positive. The major fatty acids were iso-$C_{15:0}$ and iso-$C_{15:0}$ 2-OH. The major polar lipids were one aminophospholipid (APL1), two aminolipids (AL1, AL2) and three unknown lipids (UL1, UL2, UL3). Menaquinone 7 (MK-7) was the major respiratory quinone. The DNA G+C content of strain SPSPC-11^T was 39.2 mol% by HPLC method; 37.6 mol% by genome sequencing. The high quality draft genome of the organism will also be mentioned, particularly relating to some aspects of the corroboration of phenotypic characteristics. Based on phylogenetic, physiological and biochemical characteristics we describe a new species of a novel genus represented by strain SPSPC-11^T (=CECT 9012^T =LMG 29233^T) for which we propose the name *Raineya tepidiphila* gen. nov., sp. nov. We also propose the family *Raineyaceae* to accommodate this new genus.

P 17 EVALUATION OF ANTITUBERCULOSIS ACTIVITY OF AROMATIC EXTRACTS FROM SOME BULGARIAN OLEAGINOUS PLANTS

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Medicinal plants have been used for treatment of diseases since the early civilizations. A large number of them contain essential oils that have extensive bioactivities as antimicrobial agents.
In this study 8 aromatic extracts from some Bulgarian oleaginous plants were tested for their *in vitro* antimycobacterial activity against reference strain *Mycobacterium tuberculosis* $H_{37}H_v$ using the resazurin colorimetric microplate assay. Each oil was tested at concentration diapason since 5 mg/ml to 0.1 mg/ml. Ethambutol and isoniazid were used as positive controls. The results are present as minimal inhibitory concentrations (MICs, mg/ml) of different oils using bacterial suspensions (10^5cells/ml) prepared directly from solid media.
Evaluation of the antimycobacterial activity of the essential oils of Bulgarian *Lolosevich* and *Rosa alba* L., as well absolue of *Rosa alba* L. and *Rosa damascena* Mill. identified MIC values equal to the controls 0.2 μg/ml. MIC values lower then 0.2 μg/ml are ideal candidates against *M. tuberculosis* agents and therefore *Rosa damascene* Mill. can be considered to possess highly antimycobacterial activity with MIC 0.1 μg/ml.
Therefore the results of this study support the use of this essential oils as an alternative remedy for treatment and prevention of tuberculosis, as well as an inhalation form in anti-tuberculosis therapy, where they are able to exert not only local, but also systemic effects.
These aromatic extracts would be best choice for further preclinical and clinical studies as effective agents against *Mycobacterium tuberculosis* disease.

P 18 ESSENTIAL OIL OF *ROSA ALBA* L. AS ANTIOXIDANT WITH FINE FLAVOR, AND LOW CYTOTOXIC AND GENOTOXIC EFFECT

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Chromatographic profile, antiradical properties of *Rosa alba* L. essential rose oil, and investigation of its cytotoxic/genotoxic effect on phylogenetically distant test-systems - *Hordeum vulgare* L. and human lymphocytes *in vitro* were presented in this work.
Essential oil was prepared by hydrodestilation and analyzed by GC/MC. Cytotoxicity of rose oil was evaluated using mitotic index. Endpoints for genotoxicity were chromosome aberrations and micronuclei. Barley root tip meristems were treated with 250 - 1000 μg/ml oil and lymphocytes were treated with 50–500 μg/ml, respectively. Untreated cells were used as a negative control.
Geraniol was the most abundant compound in *Rosa alba* L. essential oil (18.28 %), being citronellol (18.00 %). Its potential to scavenge DPPH radicals (IC_{50} = 2.1 $\mu g.L^{-1}$) was higher them ascorbic acid and butylated hydroxyl toluene. Weak cytotoxic effect was obtained in barley and human lymphocytes in a concentration range used in the present study. Well-expressed genotoxic activity dependending on concentration was found after treatment with rose oil evaluated as frequency of

chromosome aberrations and micronuclei in both test-systems. Data obtained suggest the possibility for application of *R. alba* L. oil as reliable pharmaceutical therapeutic.

P 19 SEDIMENT BACTERIAL COMMUNITIES ALONG HEAVY METAL GRADIENT IN SMALL HYDROPOWER PLANT CASCADE

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Heavy metals are widespread pollutants with significant environment risk due to high toxicity and tendency for accumulation in different matrices – soils, sediments, biota. The main research objective of this work is to assess the impact of heavy metal pollution on key structural and functional parameters of microbial communities in sediments of small hydropower cascade Middle Iskar, Bulgaria. The content of heavy metals (As, Cd, Cu, Hg, Pb, Zn) was measured during the low water summer periods of 2012, 2013 and 2014. The evaluation of site quality and heavy metal pollution was done by use of one integrated index - Pollution Load Index (PLI) and it was compared to total count of sediment microbiota and count of coliform bacteria, also with total dehydrogenase activity and index of phosphatase activity. The assessment of heavy metal pollution in river-dam sequence of cascade indicates the higher metal concentrations and high PLI in dam sediments. At low level of pollution in river sites, the both structural and functional microbial parameters react to local variations of heavy metal concentrations and high negative correlation ($r=-0.8 \div -0.9$) exist between variables. But in dam sites, the microbial community is more resistant to pollution and structural parameters react conservatively with long reaction time. The enzyme activities are more adaptive and sensitive indicators for different level of environmental impact in this case. The complex phosphatase and dehydrogenase activities have a high potential to be used as reliable parameters for precise assessment of hazardous sediment pollution in complicated ecological situations with cumulative impacts.

Keywords: sediment bacterial community, heavy metals, dehydrogenase activity, phosphatase activity, small hydropower cascade

P 20 MULTIFUNCTIONAL ENZYMES RELATED TO SIALIC ACID CLEAVAGE AND TRANSFER IN MICROORGANISMS

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Cleavage and transfer of sialic acid residues are key steps in microbe-host interactions for microorganisms that exist in close contact with most of the higher animals. Generally, these steps are catalyzed by three groups of enzymes: sialidases, sialyltransferases and trans-sialidases, each group performing a specific reaction. Recent studies reveal that some of these enzymes unite the three types of activities in a single protein molecule. This is a phenomenon of multifunctionality, which seems to be economic strategy for microorganisms to employ various substrates without expansion of genome. In practical aspect, multifunctionality contributes to some applications of these enzymes in chemical and pharmaceutical industries.

Key words: sialic acids, multifunctional enzymes, sialylation, trans-sialidases

P 22 MIMOTOPE ARRAYS FOR ANTIBODY PROFILING

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Antibody repertoires can be profiled or monitored using next generation sequencing (NGS), phage display libraries and microarrays. Put together, these methods provide a global view of the antibody reactivities using arrays of peptides. The sets of antibody binding data can be further analyzed focusing on the individual specificities or on the complex patterns they form. The structures recognized are formally mimotopes except when they are known epitopes eliciting the antibody response. The current state and the future application of mimotope arrays in disease monitoring and vaccination are discussed.
Key words: antibody repertoire, microarray, peptide, NGS, phage display

P 23 THE URGENCY OF EFFECTIVE ANTITUBERCULAR DRUG DEVELOPMENT – NEW PROMISING STRUCTURES DERIVED FROM NATURAL TERPENOIDS

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Background: Despite the availability of highly efficacious treatment for decades, tuberculosis (TB) remains a major global health problem. The widespread transmission of resistant variants of *Mycobacterium tuberculosis*, which does not respond to any of the commercial drugs, threatens health security of both developed and developing world. The urgent need of new antimycobacterial agents and development pathways is becoming more and more apparent.
Materials & Methods: More than 200 new diverse structures, including more than 50 new synthetic chiral compounds derived from natural terpenoids (+)-camphor and (-)-fenchone were synthesized. The compounds were evaluated for their *in vitro* antimycobacterial activity by proportional method of Caneti and REMA (Resazurin Microtiter Assay) against reference strain *Mycobacterium tuberculosis* H37Rv.
Results: The quantitative structure–activity relationship (QSAR) revealed several structural requirements: two hydrogen bond donors, two or three rings and no large branched substituents. We describe the design of a set of nine novel camphane-based derivatives following these requirements. Four of them showed activities in the nanomolar range, significantly higher than the activities in the initial set. Many structures showed promising antimycobacterial activity (MIC up to 0.27 M) – 10 to 20 fold higher than activity of ethambutol in combination with insignificant cytotoxicity (IC50 more than 354M toward human embryonal kidney cell line).
Conclusion: When developing new drugs against *Mycobacterium tuberculosis*, it is important to note that this bacteria has a solid and chemically resistant cell wall. Therefore, anti-TB agents are specific and do not act on other pathogenic bacteria, and vice versa - the huge variety of available antibiotics does not affect the mycobacteria. For all tested compounds there is no correlation between their antimycobacterial activity and activity against other microorganisms. This indicates that the action of all potent derivatives ((+)-camphor and (-)-fenchones) is specific to the *Mycobacterium tuberculosis*. All of them are stable, non-toxic against human cells and show antimycobacterial activity in the nanomolar range being 60 times more active than ethambutol. These results can be considered an important starting point for design of new effective antitubercular drugs.

P 24 DO THE NANODIAMONDS ALTER THE ENZYMOLOGICAL PROFILE OF BIOFILM COMMUNITY?

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Nanodiamonds (ND) are nano-sized particles known for their biocompatibility, chemical and mechanical stability and high adsorption capacity. In this study they are used as stimulating factor for azo-dye biodegradation carried out by biofilm from lab-scale down-flow sand biofilter. The activities of four key enzymes from the biodegradation pathway of Amaranth were studied (azoreductase, catechol-1,2-dioxygenase, catechol-2,3-dioxygenase, succinate dehydrogenase).

The obtained results showed that before ND addition the main azoreduction (first step of Amaranth biodegradation) was concentrated in the bottom layer of the biofilter (7.74 μmol/min.mgP). Also no catechol-1,2-dioxygenase and catechol-2,3-dioxygenase activities (key detoxification enzymes) were found. The highest succinate dehydrogenase activity in the middle layer (0.78 μmol/min.mgP) indicated that the most of the trivial organic pollutants are degraded there.

The addition of ND stimulated the reduction of Amaranth azo-bond as the highest azoreductase activity shifted in the upper layer of the biofitler (87 % increase for that layer) despite the highest concentration of the toxicant. The two alternative steps for benzene ring cleavage (catalyzed by catechol-1,2-dioxygenase and catechol-1,2-dioxygenase) were "turned on". The succinate dehydrogenase activity in the upper layer also increased (with 27 %) but the highest values remains in the middle layer.

All this showed that after ND were applied the azo-degradation activity of the microbial community was increased and the biofilm performed full degradation of the azo-dye Amaranth.

Keywords: nanodiamonds, detoxification, azo-dye, nano-stimulation, key enzyme activities

P 25 GLUCOSE EFFECT ON COLD-ACTIVE CATALASE PRODUCTION BY ANTARCTIC *PENICILIUM CHRYSIGENUM* P29

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H_2O_2 is a harmful metabolic by-product of aerobic life that also acts as second messenger in signal transduction pathways. During cellular evolution, its rapid and effective removal by various oxidoreductases, including catalase (CAT) was of essential importance. Cold-active (CA) CAT belongs to the enzymes that exhibit activity at temperatures of approx. 0-30°C**.** Novel CA CAT has great potential of application in food, textile and pharmaceutical industries, as well as in medicine. It may be produced by organisms existing in permanently cold habitats. Antarctic filamentous fungi are adapted for protection against hydrogen peroxide, which is important for survival at low temperatures where the solubility of oxygen is increased. The psychrotolerant strain *Penicillium chrysogenum* p29 was selected among 80 Antarctic isolates as a good producer of cold-active CAT. The aim of this study was to investigate the effect of glucose on CA CAT production by this model strain. The increase in glucose concentration from 1 to 5% caused an accelerated growth of the producer, followed by a plateau (5 – 8%) and then by growth retardation (9 – 10%). These changes were companied by full consumption of the substrate. Maximum intracellular CAT activity (78 E/mg protein) was detected in the medium with 2% glucose. Upon addition of 4% glucose the enzyme level

decreased to about 40 E/mg protein and remained at this level up to 10%. The extracellular activity outlined the same trend. It can be assumed that the CA CAT biosynthesis is regulated by *mechanism* of glucose *catabolite repression.*

Key words*: cold-active enzymes, catalase, Antarctic fungi, glucose, catabolite repression*

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P 26 SCREENING AND COMPARATIVE CHARACTERISTICS OF LACTIC ACID BACTERIA STRAINS FOR THE BIOSINTHESIS OF EXOPOLYSACCHARIDES

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The interest in the production of exopolysaccharides by lactic acid bacteria isolated from medicinal plants, as potential starter components for the preparation of fermented foods with improved texture properties has increased.

Screening of this study included 54 plant strains of lactic acid bacteria of the genera *Streptococcus, Enterococcus, Lactobacillus, Lactococcus,* isolated from the medicinal plants *Geranium sanguineum* L., *Panax ginseng* C.A.Meyer , *Hypericum perforatum* L. and four types of *Salvia (S. officinalis* L., *S. ringens* Sibth. & Sm., *S. blepharophylla* Brandegee ex Epling, *S. scabiosifolia* Lam.*)*. It was found that in between the examined strains of lactic acid bacteria from plant origin, active producers of exopolysaccharides were genera *Enterococcus* – 120-340 $mg \cdot L^{-1}$ and *Lactobacillus* – 100-250 $mg \cdot L^{-1}$. The biological activity of the genera *Streptococcus* and *Lactococcus*, varies in the range of 75-200 $mg \cdot L^{-1}$. A comparative profile of exopolysaccharides produced by lactic acid bacteria isolated from dairy products and belonging to the genus *Streptococcus, Lactobacillus, Lactococcus, Enterococcus* and *Leuconostoc* was made. The amount of produced exopolysaccharides reported for the dairy strains ranges between 50-250 $mg \cdot L^{-1}$. Active producers of the polymers examined were: *S. thermophilus* – 50-250 $mg \cdot L^{-1}$; *Lb. delbrueckii* subsp. *bulgaricus* – 60-150 $mg \cdot L^{-1}$; *L. lactis* subsp. *cremoris* – 25-200 $mg \cdot L^{1}$; *Lb. casei* – 50-60 $mg \cdot L^{1}$.

The study aims to identify the potential of plant-derived lactic acid bacteria for the biosynthesis of exopolysaccharides and to make a comparative profile with the appropriate representatives of milk origin.

Keywords: medical plants, lactic acid bacteria, exopolysaccharides, synthesis, properties, fermented foods.

P 27 BIODIVERSITY OF LACTIC ACID MICROBIOTA IN PLANT ECOSYSTEMS

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Medicinal plants are diverse and unexplored ecosystem for isolation of various microbial populations. Each specific plant species provides a unique environment in terms of competing microorganisms, natural plant antagonists, as well as accessibility, type and concentration of the substrate in the various physical factors. These conditions allow for the growth of typical epiphytic flora. Results for biodiversity of lactic acid bacteria isolated from medicinal plants belonging to families Lamiaceae, Geraniaceae, Araliaceae and Hypericaceae are presented for the first time. As a result of large-scale screening of 1200 microbial isolates (derived from flower, leaf or stem of 5 species *Salvia – S. officinalis* L., *S. ringens* Sibth. & Sm., *S. blepharophylla* Brandegee ex Epling, *S. scabiosifolia* Lam., *S. tomentosa* Mill.; *Geranium sanguineum* L.; *Hypericum perforatum* L. and *Panax ginseng* C.A.Meyer) based on visual assessment of milk coagulation, gas formation and non-specific odour, 188 microbial isolates (23.5 %) were selected, and they formed a solid coagulum, did not form gas and have not had non-specific odor. 154 single colonies showed phenotypic identity with the lactobacteria group, after growth on selective media (M17 and MRS) and performed required and confirmatory tests (Gram staining, catalase test, oxidase test, indole reaction,. These bacteria are homofermentative cocci and rods with a wide pH (5.0–9.6) and temperature range (15 – 45 °C), high salt tolerance (3.0 – 10.0 % NaCl), and high acid-producing activity (3.5–15.6 g/L).
Using genotype-based methods such as 16S rDNA sequencing, the plant-derived bacterial isolates were identified as: *Lactobacillus rhamnosus*, *Enterococcus faecium*, *Enterococcus casseliflavus*, *Enterococcus mundtii*, *Lactococcus lactis* and *Streptococcus thermophilus*.
Keywords: lactic acid bacteria, medicinal plants, biodiversity, properties

P 28 INNOVATIVE ECOLOGICAL APPROACHES FOR DAIRY WASTEWATER TREATMENT

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The most effective technology for dairy wastewater treatment includes an anaerobic and an aerobic bioreactor but only aerobic bioreactors were introduced in the most dairies in Bulgaria. The creation of effectively and efficiently operating anaerobic reactor with specialized biological system is one of the real requirements in the practice. The goal is by consecutively application of innovative ecological approaches to accelerate dairy wastewater treatment processes and to increase biodegradation effectiveness to target pollutants. The working hypothesis is based on following key points: i/ introduction of a comprehensive biotechnology (bioreactors; biological systems; operating parameters and system for control) for dairy wastewater treatment is a necessary for the real practice; ii/ the effective wastewater treatment technologies require highly specialized biological systems to accelerate biodegradation of target pollutants; iii/ the acceleration of biodegradation processes by application of ecological approaches is more environmental-friendly in comparison to the physical – chemical methods; iv/ the application of environmental-friendly technologies and highly specialized biological systems increases the effectiveness and economic efficiency of the technology. Anaerobic wastewater treatment processes in sequencing batch biofilm reactors are modeled in lab. Application of the complex approach including adaptation and immobilization accelerated lactose hydrolysis and biodegradation of organics (measured as COD) with 121 hours, and protein hydrolysis with 63 hours.

In the same time, biodegradation effectiveness of organics was increased with 5% and of proteins with 67%. Bioaugmentation approach significantly increased effectiveness of protein hydrolysis in the critical phases during wastewater treatment related to biofilm thinning and its destabilization.

Key words: adaptation, immobilization, bioaugmentation, dairy wastewater, anaerobic treatment

P29 INCREASE OF THE EFFECTIVENESS AND THE EFFICIENCY OF THE TECHNOLOGIES FOR BIOGAS PRODUCTION THROUGH A SYSTEM FOR FUNCTIONAL CONTROL

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According to the principles of the circular economy and the waste hierarchy pyramid if it is not possible to prevent its formation or to reuse it the waste should be treated and used as raw materials. The most convenient way to recycle the food waste, the agriculture waste, the sludge formed in the process of wastewater treatment is their treatment in the widely distributed technologies for biogas production. Every biotechnology consists of four components: an equipment, a biological system, process parameters and a control system. The critical problems in the technologies for biogas production are: 1/ a lack of adequate and express control – till this moment it has not been applied a biological functional control of the system but just external technological indicators; 2/ an unsolved task is the increase of the methane in the biogas – the biogas can be used for different energy needs depending on the quantity of methane. After large analysis we proposed two innovations solving the identified problems – a fluorescent system for indication of the anaerobic digestion as well as an ultrasonic pretreatment of the substrate in order to increase the percent of methane in the biogas. The control system uses reliable express biological indicators of the functioning of these technologies presenting the accurate condition of the biological system that could allow to adapt the microbiological consortium and could lead to the increase of the quantity of methane in the biogas, to the increase of the effectiveness and the efficiency of the technologies for biogas production. The proposed innovations will be applied in the technology for biogas production located on the territory Sofia plant of solid waste treatment "Chan Bogrov" as well as in methane tanks of Sofia wastewater treatment plant "Kubratovo".

P30 MODULATION EFFECT OF NANODIAMONDS ON DYNAMICS OF KEY GROUPS OF MICROORGANISMS IN BIOREMEDIATION OF SEDIMENTS CONTAMINATED WITH PHENOL

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In the field of microbiology increasingly find their place carbon nanoparticles, such as nanodiamonds (ND). Less affected remains a question related to their modulating action on quantitative changes of microorganisms during biodetoxification processes on different types of xenobiotics. In this study in laboratory conditions were modeled processes of biodetoxification of phenol in sediments, maximally approximated real ones. Simulated hazardous situation of loading with phenol was in the next conditions: 1/ phenol in the concentration close to the critical – 250 $mg.L^{-1}$ and in concentration three times higher than critical. In this study was used an innovative element - the application of nanodiamonds as augmentation factor, added at the start of the process. Analysis of bioremediation process was realized with integrated methodological arsenal of chemical, kinetic and microbiological

indicators. The focus of the study was put on the microbial communities and on their restructuring in parallel in the course of detoxification processes. The study included groups of aerobic and anaerobic heterotrophs, phenol-degrading bacteria and bacteria from key xenobiotic-degrading complex - *Pseudomonas* and *Acinetobacter*.

At relatively low concentrations of phenol (near to critical) ND adsorbed phenol and created clusters in which were concentrated microorganisms, enzymes and phenol. This was associated with an increasing of the amount of aerobic and anaerobic heterotrophs and bacteria from p. *Acinetobacter*. In explosive load with phenol microbial communities of xenobiotic-degrading complex were restructured and the increase of the amount of pseudomonads and anaerobic heterotrophs were registered. ND as augmentative factor enhanced the adaptation of microbial societies and their biodegradation activity to toxic pollutants. The augmentative effect was higher in the risk situations when phenol was in shock high concentration.

Keywords: biodetoxification, sediments, microbial societies, phenol, nanodiamonds

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