Prof. Hristo Najdenski, DVM, DSc, Corresponding Member of BAS

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Prof. Nina Ivanovska, DSc

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Assoc Prof. Zlatka Alexieva, PhD

Chairman of Scientific Council
## CONTENT

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scientific Reports on Projects and Publications</td>
<td>5</td>
</tr>
<tr>
<td>Departament of General Microbiology</td>
<td>6</td>
</tr>
<tr>
<td>Departament of Infectious Microbiology</td>
<td>8</td>
</tr>
<tr>
<td>Departament of Applied Microbiology</td>
<td>13</td>
</tr>
<tr>
<td>Departament of Virology</td>
<td>22</td>
</tr>
<tr>
<td>Departament of Immunology</td>
<td>29</td>
</tr>
<tr>
<td>Section of Mycology</td>
<td>33</td>
</tr>
<tr>
<td>List of Publications not included in the Scientific Projects</td>
<td>37</td>
</tr>
<tr>
<td>Education Activity</td>
<td>43</td>
</tr>
<tr>
<td>Scientific Events</td>
<td>47</td>
</tr>
<tr>
<td>Staff of the Stephan Angeloff Institute of Microbiology</td>
<td>50</td>
</tr>
<tr>
<td>Scientific Board</td>
<td>53</td>
</tr>
<tr>
<td>International Scientific Board</td>
<td>54</td>
</tr>
<tr>
<td>National Scientific Seminars</td>
<td>55</td>
</tr>
<tr>
<td>Annex 1 - Photos of Important Events</td>
<td>56</td>
</tr>
</tbody>
</table>
SCIENTIFIC PROJECT REPORTS
PUBLICATIONS
ACTIVITIES
DEPARTMENT OF GENERAL MICROBIOLOGY

SECTION OF MICROBIAL GENETICS

Current projects

MODERN MOLECULAR APPROACHES FOR FUNCTIONAL CHARACTERIZATION OF THE PROBIOTIC PROPERTIES AND TECHNOLOGICAL CHARACTERISTICS STUDY OF THE AMYLOLYTIC LACTIC ACID BACTERIA FROM BULGARIAN FERMENTATIVE PRODUCTS

Project leader: V. Gotcheva, PhD, University of Food Technologies, Plovdiv, Bulgaria

Project collaborators: The Stephan Angeloff Institute of Microbiology, BAS, Sofia; Institute of Chemical Engineering, BAS, Sofia

Research staff: A. Angelov, PhD, G. Blagoeva; P. Stefanova, P. Petrova, PhD, P. Velikova, A. Stoyanov, PhD; K. Petrov, PhD; F. Tsvetanova; L. Popova; E. Vassileva, PhD

Amylolytic lactic acid bacteria (ALAB) are diverse group of microorganisms that are capable to degrade starchy materials and to convert them directly into sugars with lower molecular weight, lactic acid, and volatiles. They have numerous applications in food industry due to specific ability to improve the organoleptic properties and to increase the nutritional value of starchy sources foodstuff in course of fermentation processing, and are also employed in perspective biotechnologies for lactic acid production using renewable and abundant starch biomass as a feedstock. During the current project, 100 strains of ALAB were isolated from amylaceous fermented foods, cereals and beverages. Twenty five new ALAB strains, belonging to 11 distinct species of four genera were isolated and analyzed. Among them, the first amylolytic Lactobacillus sakei, Enterococcus faecium and E. durans were reported. The presence and expression of the genes amyl, glgP, glgB, agl, malL, treC, and dexC were examined. Although all strains possessed extracellular and cell-bound amylase activity and produced lactic acid from starch, high genus and species specificity in the gene expression was observed. ALAB strains of genus Lactobacillus (except L. sakei) and P. acidilactici own and express all the tested genes, while E. faecium and E. durans strains expressed predominantly the gene, encoding amylase. The co-transcription of glgP and glgB genes indicates that glycogen synthesis and starch degradation occur in parallel, which is another example for dual metabolic role of biochemical paths.

Other purpose of the current project was the elucidation of probiotic-prebiotics interaction. Two β-fructosidases of L. paracasei strains (involved in inulin and oligo-fructose metabolism) were overproduced, purified and compared. The analysis of their biochemical properties showed similar pH, temperature optimum and stability. Substrate specificity revealed
that InuB41 had the highest affinity to short-chain inulin, while InuLC1 possessed the highest affinity to levan. Inulin degradation profiles of the enzymes showed the presence of fructose and sucrose only, thus proving the enzymes affiliation to exo-inulinases (EC 3.2.1.80). Highly effective bio-process for lactic acid (LA) production by simultaneous saccharification and fermentation (SSF) of chicory flour was developed. In batch fermentation with optimized medium content and fermentation conditions, a complete conversion of 136 g/L chicory flour into 123.7 g/L LA was achieved. These yield and conversion rates are the highest obtained by SSF for LA production from inulin.

Grant DFNI B02/27-2014, National Science Fund

References

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF LACTOBACILLUS STRAINS FROM UNIQUE HOMEMADE BULGARIAN FERMENTED MILK PRODUCTS

Project leader: P. Petrova, PhD

Project collaborators: Institute of Chemical Engineering, BAS, Sofia

Research staff: P. Velikova; A. Stoyanov, PhD; R. Eneva, PhD; E. Kroumova, PhD; J. Miteva-Staleva, MS; M. Zaharieva, PhD; K. Petrov, PhD; L. Popova; H. Najdenski, DSc

The aim of the project is to investigate the microbial diversity of artisanal fermented milk products from isolated and remote territory. The selection of the available strains, belonging to Lactic acid bacteria and their genetic and biochemical characterization are in progress.

Grant from the Bright Dairy & Foods Co. Ltd, China.
LABORATORY OF ZOONOZES AND BACTERIAL VIRULENCE

Current project:

DETECTION OF YERSINIA ENTEROCOLITICA IN PIG FAECES BY USING LOOP MEDIATED DNA AMPLIFICATION

Project coordinator: H. Najdenski, DSc

Collaborators: Unit “Food Safety”, Institute for Agriculture and Fisheries Research (ILVO), Melle, Belgium

Research staff: M. Zaharieva, PhD; M. Gatzovska, MS; E. van Collie, PhD; M. Heyndricks, PhD; L. Herman, PhD

Yersiniosis is the third most commonly reported zoonosis in the European Union. Consumption of contaminated pork meat is important transmission route. Current detection problems are related to the difficult processing of meat samples and time consuming bacteriological methods. Detection methods based on conventional PCR end-point analysis are faster but low sensitive. Aim of our study was to develop fast, simple, low-cost but specific and sensitive procedure for identification of Yersinia enterocolitica in pig faeces by using loop mediated DNA amplification (LAMP).

Two primers’ sets, complementary to the genes gyrB and phoP were tested for selectivity on 6 pathogenic Y. enterocolitica strains (serotypes O:3, O:5, O:8, O:9), 10 Yersinia spp. strains and 16 other Gram (-) and Gram (+) bacterial species. The sensitivity of LAMP was estimated by amplifying of serial dilution of pure bacterial DNA isolated according to the protocol of Flamm (1984) from the well characterized Y. enterocolitica strain IP684 (O:8). Five different kits for DNA isolation from tissue samples were compared. Finally, two kits for DNA - QIAamp DNA Stool Mini Kit (Qiagen) and DNA Stool isolation kit (EURx) were used for the investigations. Artificial contamination of farm samples was performed in order to optimize the reaction conditions and to evaluate the sensitivity of LAMP methodology. The products of the LAMP reaction were visualized with hydroxynaphthol blue and DNA electrophoresis in agarose gel. The sensitivity of LAMP was compared to that of TaqMan-qPCR and classical CFU enumeration on agar plates after artificial contamination of fresh pig faeces.

The primer set targeting phoP showed selectivity only for Y. enterocolitica strains. The sensitivity of LAMP was equal to that of TaqMan-qPCR – 1÷10 DNA copies per reaction after pure DNA input and 1.6x10⁴ cfu/mL after artificial contamination. The detection limit depends strongly on the DNA isolation kit used, whereby the DNA Stool isolation kit (EURx) was determined as most sensitive. The hydroxynaphthol blue visualisation corresponded to the gel electrophoresis result. In conclusion, the LAMP method is reliable, fast, cheap,
selective and sensitive, whereby the sensitivity is directly related to the DNA isolation method used. It could be applied for direct identification of food-borne pathogens based on colour visualization of the reaction product after appropriate optimizations of the reaction conditions.

Antibacterial, Radical-Scavenging and Acetylcholinesterase Inhibitori Activity of the Plant Species Solanum Carense and Geigeria Alata Used in Sudanese Folk Medicine

Project supervisor: G. Momekov, PhD, Department of Pharmacognosy, Faculty of Pharmacy, University of Sofia

Project leader: H. Najdenski, DSc

Collaborators: The Stephan Angeloff Institute of Microbiology, BAS; Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia

Research staff: M. Zaharieva, PhD; R. Gevrenova, PhD; D. Zheleva, PhD; V. Balabanova-Buzushka, PhD

Solanum carense and Geigeria alata are traditional plants used in Sudanese folk medicine for treatment of diabetes, cough, epilepsy, intestinal complaints or infections. In the frame of this project both plant species were chemically analyzed and evaluated for their antioxidant and antimicrobial potential.

A newly discovered Nε-feruloyl lysine (1) together with 16 known hydroxycinnamic acid amides (HCAAs) were identified in the roots of Sudanese plant Solanum schimperianum Hochst (syn. Solanum carense Dunal, Solanaceae). The structure of 1 was established on the basis of 1D (1H, 13C NMR) and 2D (COSY, HSQC) NMR analysis, together with UV-Vis, HR-ESI-MS and ESI-MS2 fragmentation analyses. The 16 HCAAs were analyzed using liquid chromatography coupled to mass spectrometry in parallel-reaction monitoring (PRM) mode (Orbitrap ThermoScientific). Eight HCAAs including agmatine and cadaverine amides, as well as Nε-feruloyl lysine, and sinapoyl putrescine were reported in genus Solanum for the first time.

Phenolic acids profiles of the methanol-aqueous extracts from G. alata roots and leaves were obtained by HR-ESI/MS. Major compounds of G. alata roots were isolated using low-bar liquid chromatography. Quantitative analysis of phenolic acids was performed by validated HPLC-UV with limits of detection ranged from 0.04 µg/ml to 0.57 µg/ml. For the first time protocatechuic, caffeic, p-coumaroylquinic, caffeoylsinapoylquinic, caffeoylferuloylquinic, three feruloylquinic, six caffeoylquinic acids, and caffeic acid hexoside were analyzed by HR-MS in G. alata roots and leaves. HPLC-UV determination revealed the highest content of 3,5-dicaffeoylquinic acid (25.96±2.08 mg/g dry weight) in roots, while 4,5-dicaffeoylquinic acid was the main compound in leaves (8.99 ± 0.56 mg/g dw). In total, the amount of caffeoylquinic acids reached up to 6.22% in G. alata roots.

Free radical scavenging DPPH, ABTS and ferric reducing power (FRAP) methods were used for antioxidant activity evaluation of Nε-feruloyl lysine, a hydromethanolic extract of S. shimperianum roots and G. alata roots and leaves extracts. The antimicrobial activity was estimated on a panel of pathogenic bacteria and fungi by the broth microdilution method (BMD).
N-feruloyl lysine demonstrated stronger DPPH (IC$_{50}$ 85.83 μg/mL), ABTS (IC$_{50}$ 255.54 μg/mL) and FRAP (0.44±0.02 mM TE/mg dw) as compared with the total S. schimperianum extract. The total S. schimperianum extract showed antimicrobial activity towards S. pyogenes, Listeria monocytogenes and Candida albicans (MIC 1.25 mg/ml). N-feruloyl lysine exhibited significantly higher antimicrobial activity against both Streptococcus aureus strains NBIMCC 3359 (MIC/MBC 112.5 μg/ml ) and penicillin resistant ATCC 6538 P (MIC 112.5 μg/ml) than the total S. schimperianum extract (MIC 1.25 and 2.5 mg/ml, respectively).

The 3,5-dicaffeoylquinic acid isolated from G. alata demonstrated stronger radical scavenging activity and reducing power compared to the crude G. alata extracts and pure chlorogenic acid (reference control). The 3,4,5-tricaffeoylquinic acid revealed higher antibacterial potential against the penicillin sensitive and resistant S. aureus strains, as well as towards MRSA as compared to the crude G. alata extracts and 3,5-dicaffeoylquinic acid.

In conclusion both species represent new powerful source of biological active substances with strong antioxidant potential of certain compounds and selective antimicrobial activity against Gram-positive bacterial pathogens.

“Grant-2015” of the Council for Medical Science, Medical University of Sofia

PREVALENCE OF HUMAN PATHOGENIC YERSINIA ENTEROCOLITICA IN TONSILS OF SLAUGHTER-AGED PIGS

Project supervisor: H. Najdenski, DSc

Project collaborators: Unit “Food Safety”, Institute for Agriculture and Fisheries Research (ILVO), Melle, Belgium

Research staff: M. Gatzovska, Ms, M. Zaharieva, PhD, I. Tsvetkova, Ms, L. Dimitrova, PhD student

The aim of the study was to collect preliminary data concerning the prevalence of human enteropathogenic Yersinia on the tonsils and feces of slaughtered pigs in Bulgaria and to determinate the regional distribution of these pathogens. Tonsil samples of 104 fattening pigs, as well 45 feces samples were collected at a slaughterhouse near Kostinbrod, Bulgaria.

Modified Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica was used. Two days enrichment in Peptone Sorbitol Bile Salt broth at 26°C was applied, followed by streaking on Selective Cefsulodin-Irgasan-Novobiocin (CIN) agar after alkali treatment. Typical colonies were confirmed by biochemical methods and DNA isolation of crude cell lysates was carried out. For the PCR identification specific primers for 16SrDNA and chromosomal ail gene for Y. enterocolitica and Y. pseudotuberculosis were applied (according Lambertz, 2008). This study was completed by bioserotyping of isolated Yersinia strains in Yersinia Reference Center at Pasteur Institut (Paris, France).

Of the tested pigs 20,19% were found positive for Yersinia enterocolitica of which 7.69% were proved as pathogenic Y. enterocolitica biotype 4, serotype O:3, lysotype VIII. The percentage of positive pigs of human Y. enterocolitica pathogenic strains between Stara Zagora’s and Sofia’s regions show no difference. No bacteria from the species Y. pseudotuberculosis were detected. In conclusion, the study confirms the prevalence of human enteropathogenic Y. enterocolitica as a hazard and potential risk for cross-contamination and likewise for food safety and human health. At slaughter level classical tonsils detection of human...
Pathogenic *Y. enterocolitica* should be completed by more sensitive molecular methods.

**PARATUBERCULOSIS IN ANIMALS AND HUMANS – ACTUAL HEALTH AND ECONOMIC PROBLEM**

*Project supervisor:* T. Savova, PhD, National Diagnostic and Research Veterinary Institute “Prof. G. Pavlov”

*Project leader:* M. Bonovska, PhD

*Project collaborators:* The Stephan Angeloff Institute of Microbiology, BAS; National Diagnostic and Research Veterinary Institute “Prof. G. Pavlov”, Sofia, Bulgaria

*Research staff:* H. Najdenski, DSc; V. Valcheva, PhD

*Mycobacterium avium* subspecies *paratuberculosis* is the etiological agent of paratuberculosis in animals (Johne’s disease) and in humans (Crohn’s disease). For more than three decades, official veterinary medical statistics in Bulgaria does not include data on the prevalence of paratuberculosis in animals due to its rare notification. There are no investigations and National program for control and monitoring of this disease in both domestic and wild animals. Nevertheless, several cases of paratuberculosis in a dairy farm were registered in 2014 in Northeastern Bulgaria. Diseased cows demonstrated diarrheal syndrome, birth of non-viable calves or presence of postnatal complications, severe dehydration and death. Furthermore, in recent years, the importation of cattle from other countries has increased significantly and therefore early and reliable diagnosis and the epidemiological status concerning this zoonotic disease in our country is imperative. 50 samples from wild animals (shot and dead deer and mouflons, grown freely in a hunting area in the Sofia region, Bulgaria) were obtained in the period 2009-2013. Pathomorphological lesions were observed in 4 mouflons and 3 deer with hyperplastic changes in the small intestine (bold, strongly folded and soft intestinal wall, with hyperemia). Histological analysis revealed the proliferative inflammation of the mucosa, granulomas without caseousation, macrophages with frothy cytoplasm and large number of epithelioid cells in the absence of giant cells. The microscopical, bacteriological and molecular tests show the presence of *Mycobacterium avium* subspecies *paratuberculosis*. These results indicate the need of systematic study on the prevalence of this disease in domestic and wild animals in Bulgaria. That is why the application of combination of rapid methods will allow developing of methodology for control of paratuberculosis in Bulgaria, to conduct epidemiological analysis and to assess the risk to animals and humans.

Joint research project between NDRVI and SAIM, BAS

LABORATORY OF ANTIMICROBIAL AGENTS

Current project

WATER-SOLUBLE PHTHALOCYANINES FOR FLUORESCENCE DIAGNOSIS AND PHOTODYNAMIC THERAPY

Project supervisor: M. Durmuş, PhD, Gebze Institute of Technology, Turkey

Project leader: V. Mantareva, PhD, Institute of Organic Chemistry with Centre of Phytochemistry, BAS

Project collaborators: The Stephan Angeloff Institute of Microbiology, BAS; Gebze Institute of Technology, Turkey; Institute of Organic Chemistry with Centre of Phytochemistry, BAS

Research staff: V. Kussovski, PhD, I. Angelov, PhD

Two recently synthesized water-soluble cationic lutetium (III) acetate phthalocyanines (LuPc-5 and LuPc-6) were studied as photosensitizers for antimicrobial photodynamic therapy towards two pathogenic microorganisms, namely the Gram-negative bacterium Pseudomonas aeruginosa and a fungus Candida albicans. The photodynamic effect was evaluated for the cells in suspensions and organized in 48-h developed biofilms. The relatively high levels of uptakes of LuPc-5 and LuPc-6 were determined for fungal cells compared to bacterial cells. The penetration depths and distribution of both LuPcs into microbial biofilms were investigated by means of confocal fluorescence microscopy. The photoinactivation efficiency was studied for a wide concentration range (0.85–30 μM) of LuPc-5 and LuPc-6 at a light dose of 50 J cm−2 from red light-emitting diode (LED; 665 nm). The full photoinactivation was achieved with 20 μM LuPc-5 for P. aeruginosa and 30 μM LuPc-5 for C. albicans. The PDI study on microbial biofilms showed incomplete photoinactivation (<3 logs) for the used gentle drug-light protocol.

Grant No: 212M053 Joint research project TUBITAK (Turkey) – BAS (Bulgaria)

References:
Final Summary

BIOTECHNOLOGICAL AND ECO-FRIENDLY APPROACH FOR EFFECTIVE UTILIZATION OF WASTE PLANT BIOMASS FOR COMPOST AND SOIL IMPROVEMENTS

*Project leaders*: A. Gousterova, PhD and K. Tsekova, PhD  
*Project collaborators*: Inovet OOD, Sofia  
*Research staff*: H. Najdenski, DSc; D. Todorova, PhD; D. Paskaleva, MS; I. Tzvetkova, MS

During the fulfillment of the activities by the project the main set purpose was achieved, and namely deriving of cheap and harmless plant bioproducts /compost and soil improver/ from plant waste. For this purpose were made industrial scientific researches for developing of methods for more effective utilization of these waste products, based on enzyme hydrolysis in using of mixed culture of selected termophile microorganisms. The microbiological and the biochemical processes, which pass during the composting of the plant waste, were studied. The biological activity of the ready product (compost) and its components, the content of heavy metals and presence of pathogenic microflora were researched. In addition during the fulfillment of the present project all indicators were achieved, which were set in the contract, and namely:

Grant BG161PO003-1.1.06 from the Operational Programme “Development of the Competitiveness of the Bulgarian Economics”, EU, European Regional Development Fund.

Current projects

CULTIVATION OF MICRO-ALGAE IN INNOVATIVE PHOTO-BIOREACTOR FOR CARBON DIOXIDE SEQUESTRATION FROM INDUSTRIAL WASTE GASES FROM DIFFERENT SOURCES AND SYNTHESIS OF HIGH VALUE PRODUCTS

*Project leader*: A. Kroumov, PhD, The Stephan Angeloff Institute of Microbiology, BAS  
*Project collaborators*: West Parana State University, Department of Chemical Engineering, Brazil  
*Research staff*: A. N. Módenes; D. E. G. Trigueros; F. R. Espinoza-Quiñones; C. E. Borba; F. B. Scheufele; C. L. Hinterholz

Theoretical achievements included development of the novel strategy for column photobioreactors (PBRs) modeling, optimization, design and scale-up. The novel
strategy was presented in several symposia and congresses and now is in progress of publication in Process Biochemistry Journal.

Set up of the Algae Lab. Several stands for performing algal culturing techniques were set up according to the principles of this strategy. They included (PBRs) section as follows: section with 16 small PBRs; section for flat plate PBRs; section for maintaining isolated strains.

Several algal strains were isolated from Brazilian lakes and preliminary morphological identification was performed. Their identification is in progress by using modern molecular DNA methods.

EFFICIENT USE OF BIOMASS FOR ENERGY AND ENVIRONMENTAL OBJECTIVES: THE POTENTIAL OF BIOETHANOL AS A FUEL FOR RAW MATERIALS

Project leader: S. D. Ivanova, PhD, Institute of Catalysis, BAS

Research staff: A. Kroumov, PhD

Project collaborator: The Stephan Angeloff Institute of Microbiology, BAS; Institute of Catalysis, BAS

Modeling the process of ethanol production from lignocellulosic wastes (LCW), for example, wheat straw. The model considered simultaneous enzymatic hydrolysis of LCW and ethanol synthesis from the hydrolysis products.

Structure of the model. The author's work was focused on enriching our knowledge about the process of simultaneous saccharification of lignocellulosic waste (SSLCW) and the production of ethanol. The model was used to studying the relationships between state parameters and subsequent planning of active experiments. The simulation results helped to discriminate different working hypotheses and accelerate the search of finding optimal operating conditions of the process.

The development of this model is a continuation of multiannual work of the author in the field of process modeling (laboratory and industrial experiments) of ethanol production based on different substrates, reflected in his famous 10 publications.

Grant E02/1, National Science Fund

References:
ANAEROBIC BIODEGRADATION OF LIGNOCELLULOSIC WASTE TO PRODUCE BIOMETHANE AND UTILIZATION OF CO₂ FROM IT BY USING MICROALGAE

Project leader: A. D. Kroumov, PhD

Theoretical achievements included development of the novel strategy for column photobioreactors (PBRs) scheme and their modeling, optimization, design and scale-up applied for CO₂ fixation from biogas. The purification scheme included two PBRs in series and gas holder where simulated biogas was loaded. The novel strategy was presented in several symposia and congresses and now is progress of publication in Process Biochemistry Journal.

Grant DFNI-E01/0001, National Science Fund

References:

OPTIMIZATION OF FERMENTATION PROCESSES FOR AMINO ACID PRODUCTION

Project leader: A. Ratkov, PhD

Research Staff: I. Dimov, MS; R. Prtkova, MS; F. Filipov, MS; J. Kristeva, MS

Caring of fermentation processes for microbial production of different amino acids (mainly lysine, valine and leucine) using different mutant strains as a producers. Optimization of fermentation processes based on specifics substrate requirement of the producers as well as technological and fisico-chemical optimization of the processes for their production.

Bilateral Agreement for Cooperation between The Stephan Angeloff Institute of Microbiology, BAS and RTM “Recourses+Technologies Management”, Germany

CARRYING OF TEST AND DEMONSTRATION EXPERIMENTS

Project leader: Alexander Ratkov
Research Staff: I. Dimov, MS; R. Prtkova, MS; F. Filipov, MS; J. Kristeva, MS

Test experiments included evaluation of specific main raw materials for microbial production of L-lysine. Testing is focused on the specificity and the efficiency of predicted for industrial production main C-source and main protein source. Based on the specific main raw materials the detailed technological regalement for production of feed grade lysine sulfate as a final product is developed. Education of the Russian staff is predicted.

Bilateral Agreement for Cooperation Project between Institute of Microbiology, BAS and Company “Plemzavod-Jubileinie”, Russian Federation.
LABORATORY OF EXTREMOPHILIC BACTERIA

Current projects

BIODIVERSITY OF HALOPHILIC MICROORGANISMS ISOLATED FROM BULGARIAN SALT NICHES AND THEIR EXOPOLYSACCHARIDE SYNTHESIS POTENTIAL

Project supervisor: M. Kambourova, DSc
Project collaborators: University of Food Technologies
Research staff: E. Tonkova-Vasileva, PhD; N. Radchenkova, PhD; I. Boyadzhieva, PhD; S. Vasilev, MSc

Diversity of halophilic microorganisms in halophilic niches from the southern Black Sea coast was studied by unculturable and culturable approach. Investigations on the archaeal community composition in P18, the biggest crystallizer pond in Pomorie salters (PS) (34% salinity) demonstrated unusually high number of presented taxa in hypersaline environment. Archaeal clones were grouped in 26 different operational taxonomic units (OTUs) assigned to 15 different genera from two orders, Halobacteriales and Haloferacales. New sequences represented 53.9% of archaeal OTUs. Present results significantly differed from the previous investigations in regard to the number of presented genera, the domination of some genera not reported before in such extreme niche and the identification of previously undiscovered 16S rRNA sequences.

Culturable approach revealed high for such niches taxonomic and metabolic diversity: we isolated 19 moderate halophilic and 10 halotolerant strains affiliated with 12 species from eight genera referred to the phyla Proteobacteria, Firmicutes and Actinobacteria. Isolates were determined as alkalitolerant, and 41% of them as psychrotolerant. Half of the tested 18 substrates were degraded by isolates, with predominance of producers of polygalacturonase, catalase, phytase and lipase. More than a half of the strains (65%) displayed antimicrobial activity against one to five model bacteria and yeasts.

Grant DFNI 02-26/2014, National Science Fund

References:
CHARACTERIZATION OF ARCHAEAL DIVERSITY IN BULGARIAN HOT SPRINGS

Project supervisor: Assoc. Prof. M. Kambourova, DSc

Project collaborator: Department of Biology and Centre for Geobiology, University of Bergen, Bergen, Norway

Research staff: N. Boteva, PhD student; I. Boyadzhieva, PhD; N. Radchenkova, PhD; M. Stoilova-Disheva, PhD; D. Lyutskanova, PhD; S. Vasilev, MSc

A culture independent approach based on the analysis of nearly complete archaeal 16S rRNA genes revealed an unusually high archaeal diversity in one of the hottest Bulgarian geothermal springs, Vlasa, Velingrad (86°C). Archaeal clones were referred to 30 OTUs. They were affiliated with both archaeal kingdoms, Euryarchaeota and the provisional Proteoarchaeota, with a predomination of proteoarchaeal clones. Representatives of five among seven currently provisional proteorarchaeal phyla were identified in the sample - Crenarchaeota, Thaumarchaeota, Aigarchaeota, Korarchaeota, and Geoaarchaeota. All sequenced clones showed closest similarity to uncultured representatives from geothermal niches and some of them formed separate branches. More than two third of the retrieved sequences were >3 % different from their closest matches, suggesting the presence of representatives of novel species, and even genera in this niche. Four of them differ from the closest relatives with more than 15%, the border for new phyla. More than one third of them are unclassifiable, as the sequence similarity to the closest classified relative in databases is lower than 80%. Low similarity with cultured archaeal representatives and low match to environmental 16S rRNA clones are unique features for this thermophilic niche, indicating that the archaeal diversity is still underexplored. The sequences retrieved from the Vlasa hot spring demonstrate that this environment harbors one of the richest archaeal communities found and contribute to expanding our knowledge of archaeal diversity in geothermal environments.

EEA Program, Grant D03-100/05.06.2015

Reference:

New project

EXOPOLYSACCHARIDE FROM HALOPHILES: PRODUCTION, CHEMICAL CHARACTERIZATION AND THEIR POSSIBLE BIOTECHNOLOGICAL APPLICATIONS

Project supervisor: M. Kambourova, DSc

Project collaborator: Institute of Chemistry of Biomolecules, CNR, Naples, Italy

Research staff: N. Radchenkova, PhD; I. Boyadzhieva, PhD
LABORATORY OF APPLIED BIOTECHNOLOGIES

Current projects:

RATIONAL PLATFORM FOR SUSTAINABLE PRODUCTION OF PHARMACEUTICALLY RELEVANT MOLECULES FROM PLANTS AND THEIR IN VITRO CULTURE

Project leader: M. I. Georgiev, PhD

Project collaborators: Institute of Organic Chemistry with Centre of Phytochemistry-BAS; University of Sofia

Research staff: P. Dimitrova, PhD; A. Marchev, PhD; S. Rusinova-Videva, PhD; V. Milanova, Georgi Zahmanov, Elka Genova

Chronic (systemic) inflammation and malignant neoplasms are socially-significant diseases, which affect millions of people worldwide. Chemotherapeutic agents currently in use in the clinics did not succeed in fulfilling their expectations even though they are very cost-intensive. In parallel, there is an increasing evidence for the potential of plant-derived secondary metabolites on the inhibition of different steps of tumorigenesis and associated inflammatory processes, underlining the importance of these products in cancer prevention and therapy. The aim of the project is to entail an elaboration of a rational platform for sustainable production of pharmaceutically relevant value-added biologically active molecules from plants and their in vitro systems. The project is built strongly on scientific excellence and directly reflects to the National Scientific Research Strategy and Innovation Strategy for Smart Specialization of Bulgaria 2014-2020.

Grant DFNI B02/14, National Science Fund

References:

RATIONAL PLATFORM FOR HALOGENATION OF HIGH-VALUE PHENYLETHANOID GLYCOSIDES FROM PLANT ROOT CULTURE

Project leader: M.I. Georgiev, PhD

Project collaborators: Institute of Botany, Dresden University of Technology (Germany)

Research staff: P. Dimitrova; A. Marchev, PhD; S. Rusinova-Videva, PhD

The aim of the project (EngineeredRoots) is to set to work the expertise of the project partners in the field of plant biotechnology, molecular biology and natural products chemistry in order to elaborate a technology platform for studying
verbascoside (and concomitant phenylethanoid glycosides) halogenation in the plant cell/tissue factory. This platform includes the in vitro plant tissue culture systems (hairy roots), metabolic engineering, metabolomics and evaluation of bioactive properties of the Verbascum’s phenylethanoid glycosides. The major scientific impact of the project is on strengthening of the knowledge on bioproduction and engineering of plant-derived high-value metabolites. EngineeredRoots is built strongly on scientific excellence and it capitalizes on the strengths of Partners from the EU to deliver its objectives for maximum impact.

Grant DNTS Germany 01/8 (2014-2016)

References:

New projects

ESTABLISHMENT OF CENTRE OF PLANT SYSTEMS BIOLOGY AND BIOTECHNOLOGY FOR THE TRANSLATION OF FUNDAMENTAL RESEARCH INTO SUSTAINABLE BIO-BASED TECHNOLOGIES IN BULGARIA

Project leader: M. Georgiev, PhD

Project collaborators: University of Potsdam (Germany), Max Plank Institute of Molecular Plant Physiology (Germany), Institute of Molecular Biology and Biotechnology (Bulgaria), Maritsa Vegetable Crops Research Institute (Bulgaria)

Research staff: Andrey Marchev, PhD

The overall objective of PlantaSYST is to establish a Center of Plant Systems Biology and Biotechnology (CPSBB) in Plovdiv, Bulgaria, as a joint initiative of three Bulgarian research institutes located in Plovdiv and two institutes located in Potsdam, Germany. The ambition of CPSBB is to stand at the forefront of plant sciences in Bulgaria and Eastern Europe by integrating molecular biology, functional genomics, metabolomics, bioinformatics, bioprocessing, and long-standing expertise in practical plant genetics and breeding, to unravel the plant biology and translating the scientific knowledge into new horticultural and industrial applications. Thus, the newly established CPSBB will fill in the scientific and technological gaps identified in the Smart Specialization Strategy of Bulgaria. Besides that, the CPSBB will take a leading role in educating next-generation early-stage researchers in the fields of molecular breeding, plant systems biology and biotechnology.

Grant 664621, H2020, Teaming Spreading Excellence

References:

sexual enhancers and slimmers: the wish for better sexual well-being and perfect body can be risky. *Food Chem. Toxicol.* *(in press).*


Pending support

**COMPLEX ASSESSMENT OF DONOR GENOTYPES AND APPROACH FOR CREATION OF BULGARIAN SORTS TOMATOES WITH IMPROVED ANTIOXIDANT CONTENT AND TASTE OF THEIR FRUITS**

*Project supervisor:* B. Bojinov, PhD  
*Project leader:* A. Pavlov, DSc.  
*Research staff:* I. Ivanov, PhD; T. Gocheva; E. Genova

Grant DFNI-B01/16-1012, National Science Fund

**APPLICATION OF OMICS TECHNOLOGIES TO REVEAL HEALTH POTENTIAL OF BULGARIAN HONEY**

*Project supervisor:* M. Shishinjova, PhD  
*Project leader:* A. Pavlov, DSc.  
*Research staff:* A. Marchev, R. Vrancheva

Grant DFNI-B01/31-1012, National Science Fund
RESARCH GROUP OF MATHEMATICAL MODELLING AND COMPUTER METHODS

Current projects

NEW ECOTECHNOLOGIES FOR BIODEGRADATION OF ORGANIC WASTE WITH HYDROGEN AND METHANE PRODUCTION

Project leader: I. Simeonov, PhD

Research staff: H. Najdenski, DSc; M. Angelova, DSc; E. Chorukova, PhD; E. Krumova, PhD; V. Kasovski, PhD; L. Kabaivanova, PhD; N. Kostadinova, PhD; S. Mihaylova, MS; V. Hubenov, PhD; L. Dimitrova, PhD student

Anaerobic digestion of lignocellulosic wastes was performed with optimization to realize higher methane and hydrogen production. Chemical and biological (rep Basidiomycota) methods for pretreatment of wheat straw were tested. Comparative studies aimed at obtaining biogas from untreated and treated wheat straw in laboratory bioreactors were conducted. Comparative studies on the production of biogas from untreated wheat and barley straw in a pilot bioreactor were also performed. Mathematical models were developed for the following processes: AD with inclusion of the gas phase; A simplified model of the processes of anaerobic biodegradation of organic waste with the production of hydrogen and methane; A model of the processes for anaerobic biodegradation of organic waste to obtain hydrogen and methane based on ADM1; Method for decontamination of compost with was studied. Studies on the process of production of hydrogen from substrate cellulose were initiated.

Grant DFNI-E02/13, National Scientific Fund

ERASMUS+ (FRANCE)

Project leader: I. Simeonov, PhD

Teaching activity: I. Simeonov, PhD

Content of the teaching programme:
1. Mathematical modeling of biotechnological processes
2. Parameters and states estimation
3. Optimisation and control of biotechnological processes

Grant: European Union, Erasmus+
Current Projects:

A NOVEL APPROACH TO HIGHLY EFFICIENT CHEMOTHERAPY OF ENTEROVIRAL INFECTIONS

Project leader: A.S. Galabov, DSc
Project collaborator: M. Arita, PhD; National Institute of Infectious Diseases, Gakuen, Tokyo
Research staff: I. Nikolova, PhD; A. Stoyanova, MS; P. Grozdanov, PhD; N. Petrov, PhD; Y. Abashev, PhD; S. Philipov, PhD; N. Vilhelmova-Ilieva, PhD; L. Mukova, MS; N. Nikolova, MS; P. Stoyanova, DVM; Nikolaeva-Glomb, MD, PhD; I. Zahova; E. Dimitrova

Chemotherapy could be a leading tool for controlling enterovirus (EV) infections, but clinically effective anti-EV drugs do not currently exist, mainly due to the development of drug resistance. We were the first to investigate the combination effects of selective EV replication inhibitors in order to limit this process. In previous studies, we showed the efficacy of consecutive alternating administration (CAA) of the triple combinations disoxaril/guanidine/oxoglucine and pleconaril/guanidine/oxoglucine against Coxsackievirus B1 (CVB1) infection in newborn mice. Drug sensitivity tests of the viral brain isolates showed that these drug combinations prevented the development of drug resistance.

In the current study, we replaced guanidine-HCl with enteroviral RNA synthesis inhibitor MDL-860 to test the effect of a new triple combination - pleconaril/MDL-860/oxoglucine (PMO) - applied via CAA in newborn mice infected subcutaneously with 20 MLD50 of CVB1. The PMO combination via CAA showed high activity at the 75 mg/kg MDL-860 dose: a protective effect of 50% and a pronounced suppression of brain virus titers. Moreover, along with the prevention of drug resistance, a phenomenon of increased drug sensitivity was established. Pleconaril sensitivity increased by 7 times on Day 7 and by over 49 times on Day 13; MDL-860 sensitivity increased by 29 times on Day 7, and oxoglucine sensitivity by 6 times on Day 13. Daily, simultaneous administration of PMO showed no protective effect and a rapid development of drug resistance.

These results add new support for using CAA treatment courses to achieve clinically effective chemotherapy of EV infections.

Grant B-01-13/12, National Science Fund

References:
The synthesis of a series of more than 60 new diaryl ether compounds was accomplished starting from 2-chloro-5-nitrobenzonitrile and different phenols. The reactions were carried out under basic conditions (NaOH or KOH) in DMSO. Most of the compounds are near analogues of DNB – well known active compound against enteroviruses. All compounds were obtained in high yields and purity after column chromatography and were characterized by NMR, MS-spectra, melting points and elemental analysis.

The new synthesized diarylethers were tested for their anti-enteroviral activity against three enteroviruses: poliovirus 1 (Sabin strain) (PV1), Coxsackievirus B1 (Connectucut strain) (CVB1) and Coxsackievirus B3 (Nancy strain) (CVB3). The results obtained at the testing of the first series of 25 compounds manifested a pronounced effect of VGA-10-2 vs. PV1 and CVB1 (SI of 118, and 405, respectively). Two compounds, CB-64 and CB-69, showed a moderate activity (SI around 20) vs. CVB3. The starting compound DNB demonstrated a pronounced inhibitory effect against the three tested viruses - SI of 72.5, 586.9 and 182, respectively.

Grant Б 02-11/12.12.2014, National Science Fund
A MODERN ALTERNATIVE FOR THE PROPHYLAXIS AND TREATMENT OF INFLUENZA VIRUS INFECTION – MULTITARGET APPROACHES WITH HIGHLY EFFICIENT COMBINATIONS OF ANTIVIRAL CHEMOTHERAPEUTICS AND BIOLOGICALLY ACTIVE COMPOUNDS

Project leader: L. Simeonova, PhD

Project collaborator: Sofia University “St. Kliment Ohridski”

Research staff: E. Pavlova, PhD; L. Tancheva, PhD; G. Goujgoulova, PhD; I. Slavcheva, MS; N. Zografov, MS; G. Gegova, MS; L. Mukova, MS; K. Todorova, BS; S. Andreeva; N. Petrov, PhD

Advisors: Acad. A. S. Galabov, DM, DSc; G. Georgiev, DVM, DSc; V. Savov, DSc; M. Mileva, PhD

The necessity of efficient alternative approaches in antiinfluenza therapy continues to exist since novel antiviral inhibitors are still in an experimental phase of their development. As a strategy to combat the severity of the disease a complex inhibition of different aspects in the pathogenesis of flu could be considered, i.e. applying combinations of the available anti-influenza inhibitors and potent biological response modifiers. Beyond the cell disruptions caused by the viral replication itself, influenza infection is associated with a dramatic increase in the amount of free radicals in the organism, leading to a state of oxidative stress. Viral activation of phagocytes generates free radicals and active forms of oxygen severely damaging cellular membranes.

The hypothesis of efficacy of a combined multitarget approach was tested by standard virological, pharmacological and biophysical methods in vitro and in vivo. For the implementation of the project the following activities were performed: Antioxidant capacities of oseltamivir, ellagic acid, isprinosine were tested in cell-free model systems; toxicity of ellagic acid and protective effects of oseltamivir combined with antioxidants and immune modulator in mice against experimental H3N2 infection were established; levels of oxidative stress in experimentally infected and treated mice were investigated by measurement of enzymatic activity and was found that the oxidative levels were significantly reduced for combinations-treated groups, the effects of compounds on hepatic drug-metabolizing systems were studied.

Further study: Individual and combined effects of oseltamivir and and siRNAs in cell cultures against seasonal influenza strains: H3N2, H1N1v;

Final experiments were carried out on the combination effect of α-tocopherol and oseltamivir. Strongly dose-dependent augmented antiviral effect of the combination α-tocopherol and 0.625 mg/kg oseltamivir was demonstrated when α-tocopherol was administered simultaneously with oseltamivir: a pronounced decrease in mortality rate (a 78% protection), and a lengthening of mean survival time by 3.2–4 days. Lung parameters showed a substantial decrease in infectious virus content (∆ logs = 3.8/4.1) and a marked diminishment of lung index and pathology. Combination α-tocopherol with 1.25 mg/kg oseltamivir manifested a marked protective effect, but the effect on lung parameters was less. The combination effect of α-tocopherol with 2.5 mg/kg oseltamivir did not surpass the monotherapeutic effect of oseltamivir. When α-tocopherol was applied in courses starting 5 or 2 days before infection, its combination with oseltamivir was ineffective.

Grant B-01-19/12, National Science Fund
**BALKAN ENDEMIC NEPHROPATHY**

*Project leaders*: A. S. Galabov, DSc and M. Polenakovic, MD, DSc, Macedonian Academy of Sciences and Arts (MASA), Skopje, FYROM

*Project collaborators*: Department of Medical Genetics, Medical University of Sofia, Macedonian Academy of Sciences and Arts (MASA), Skopje, FYROM

*Research Staff*: D. Toncheva, DSc and collaborators; P. Grozdanov, PhD; I. Nikolova, PhD

The main work on the project in 2015 was the preparation of the manuscript of the monograph “Balkan Endemic Nephropathies - World Wide Diseases”. Authors of the book are from Bulgaria, Serbia and Republic of Macedonias. Editor-in-Chief is academician Vladislav Stefanovic from Serbian Academy of Sciences and Arts, Skopje, FYROM.

Academician Angel S. Galabov and Corr.-Member Draga Toncheva are among the editors. They are authors of four chapters of the monograph: „Role of Viruses in BEN” (A. S. Galabov; co-authors of the chapter B. Kamarinchev and T. Stoitsova ), „Immunologic Mechanisms” (A. S. Galabov and A. Pashov), „Genetics of BEN” (D. Toncheva and co-authors.) and “Epigenetics of BEN” (D. Toncheva and R. Staneva).

Bilateral joint project (2014-2015) in the frame of agreement between Bulgarian Academy of Sciences and Macedonian Academy of Sciences and Arts


**References:**

**BALKAN ENDEMIC NEPHROPATHY**

*Project leaders*: A. S. Galabov, DSc and V. Stefanovic, MD, PhD, Serbian Academy of Sciences and Arts (SASA)

*Project collaborators*: Department of Medical Genetics, Medical University of Sofia, Serbian Academy of Sciences and Arts (SASA), Serbia

*Research staff*: D. Toncheva, MD, DSc and collaborators; P. Grozdanov, PhD; I. Nikolova, PhD

Internet base data for patients with Balkan endemic nephropathy (BEN) is established, containing detailed clinical and pathological characteristics. The DNA bank on BEN patients for genomic and proteomic analyses was broaden. DNA from 22 patients was isolated. Ekzome analysis by NGS of 22 000 genes was done. Some
specific and general changes in BEN associated copies were identified.

The main work on the project in 2015 was the preparation of the manuscript of the monograph “Balkan Endemic Nephropathies - World Wide Diseases”. Authors of the book are from Bulgaria, Serbia and Republic of Macedinis. Editor-in-Chief is academician Vladislav Stefanovic from Serbian Academy of Sciences and Arts, Serbia.

Interacademic Bilateral Collaborative Project BAS - Serbian Academy of Sciences and Arts (2014-2015)

References:
Balkan Endemic Nephropathies - World Wide Diseases. Academician Angel S. Galabov and Corr.-Member Draga Toncheva are among the editor (see previous project).
BEN history – Zoran Radovanovich,
Tsvetan Dimitrov
Epidemiology of BEN – Zoran Radovanovich, Tsvetan Dimitrov
Etiology of BEN – Vladislav Stefanovic, Momir Polenakovic, Draga Toncheva et al.
Environmental toxins (Aristolochia, mycotoxins, polycyclic aromatic amines, others) – Vladislav Stefanovic, Draga Toncheva et al.

THE PRIMARY LIVER CANCER IN BULGARIA: HEPATITIS VIRUSES AND ENVIRONMENTAL FACTORS

Project leaders: A. S. Galabov, DSc and P. Pineau, PhD, Institut Pasteur, Paris
Research staff: L. Doumanova, PhD; P. Grozdanov, PhD
Research partners: R. Gaydarski, DSc; Tokuda Hospital Sofia; V. Dimitrova, MD, DSc, Clinics of Surgery, Alexandrovska Hospital, Sofia

The collecting of samples from patients with primary liver cancers treated surgically or undergoing the needle liver biopsy, in parallel with blood samples was continued.

ACIP Project

Role of viruses in BEN – Angel Galabov
Immunologic mechanisms – Angel Galabov, Anastas Pashov
Proteomics of BEN – Vladislav Stefanovic, Ivana Markovic, Hasan Dihazi, Guerhard Mueller
Genetics of BEN – Dragi Toncheva et al
Epigenetics of BEN - Draga Toncheva, Rada Staneva
Pathology of BEN – Ljubinka Jankovic Velickovic
Urithelial cancer – Vladislav Stefanovic, Zoran Radovanovic
Biomarkers of BEN and urothelial cancer – Liubinka Jankovic Velickovic, Vladislav Stefanovic
Diagnosis of BEN – Vladislav Stefanovic, Momir Polenakovic
Screening of BEN – Vladislav Stefanovic, Momir Polenakovic
Prevention and treatment of BEN – Vladislav Stefanovic, Momir Polenakovic
Endemic Nephropathy beyond the Balkans - Momir Polenakovic, Vladislav Stefanovic
Further research of BEN and associated urothelial cancer. What has to be done - Vladislav Stefanovic, Draga Toncheva
GENETIC PROFILE OF THE POPULATION OF BULGARIAN LANDS DURING THE THYRACIAN PERIOD: NEW PERSPECTIVES ON BULGARIAN HISTORY

**Project supervisor:** A. S. Galabov, DSc

**Project supervisor:** D. Toncheva, MD, DSc, Department of Medical Genetics, Medical University of Sofia

**Project collaborator:** D. Caramelli, PhD, University of Florence, Italy, Department of Anthropology

**Research staff:** D. Nesheva, PhD student; S. Karachanak-Yankova, PhD; P. Grozdanov, PhD; I. Nikolova, PhD

Analysis of antic DNA restored from bone residues discovered from tumuli dating from the Thracian period is done for the first time. Genetic origin of the population of Bulgarian lands from this period is determined and the contribution of this population to the contemporary Bulgarian genetic fond is estimated. 80 samples from tumuli of the 3th millennium before Christ in the regions of Stara Zagora and Sliven were studied. Isolated mtDNA (hipervariable segments I) were sequenced by the classic sequencing and the NGS analysis. The results obtained were compared to the respective areas of mtDNA of contemporary Bulgarians. Genetic profiles were compared in historical aspects.

**References:**


MODIFIERS OF BIOLOGICAL REPLY AT INFLUENZA INFECTION

**Supervizors:** M. Mileva, PhD and A.S. Galabov, DSc, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences; D. Krastev, DSc, Medical University of Sofia

**PhD Student:** A. Dimitrova, PhD student

The studies carried out show that S-adenosyl-L-methionine (SAM) has a protective effect in a model of influenza infection in mice. This substance converts in glutathione - the main antioxidant in the body, through multistep biochemical cycle. The effect of combined treatment with SAM (given as a single daily dose of 50 and 100 mg/kg in different mice groups starting from 5 days before infection until day 4 after infection) and the antiviral agent oseltamivir dose (applied at 2.5mg/kg daily in two intakes for 5 days, starting from 4 h before virus inoculation) in infected with influenza A virus mice. SAM was. Survival was 70% in the treatment with oseltamivir and raised to 90% in the treatment with oseltamivir and SAM in both of doses. SAM alone does not show any antiviral activity. The present findings suggest that therapy with molecules converted in antioxidants in the body increases survival by modulating the host
defense mechanisms and by a direct antioxidant effect against oxidative stress associated with viral infections. This study demonstrated the effectiveness of combining agents that act through different mechanisms - antiviral drug oseltamivir as specific neuraminidase inhibitor of influenza virus, and SAM as precursor of most important antioxidant - glutathione.

References:
Final Summary

FUNCTIONAL ELIMINATION OF AUTOACTIVE T CELLS BY ANTIBODY THERAPY IN MURINE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

Project leader: A. Tchorbanov, PhD

Project collaborator: J. Prechl, PhD and M. Herbáth, PhD, Immunology Research Group, Hungarian Academy of Sciences, Eötvös Loránd University

Research staff: N. Mihaylova, PhD; N. Kerekov, PhD student; S. Chausheva, graduate student; Silvya Bradyanova, graduate student; Petroslav Chipinski, graduate student

Systemic lupus erythematosus (SLE) is the prototype systemic autoimmune disease, characterized by the generation of autoantibodies specific for native (ds)DNA and nucleic acid–protein complexes, the formation of immune depositions, and inflammation in different organs and tissues. In recent years, SLE research has focused on the role of B and CD4+ T-cells in disease pathogenesis. Current therapies of the disease are non-specific and often counterbalanced by adverse effects. They are mainly based on immunosuppressive drugs such as corticosteroids and cyclophosphamide, which are administrated at high doses in phases of exacerbation.

Annexin A1 (AnxA1) (37 kDa), was originally identified as a phospholipase A2 (PLA2)-inhibitory protein and second messenger of glucocorticoid pharmacological effects. Analysis of AnxA1 expression in T cells from patients suffering from rheumatoid arthritis showed higher levels of this protein compared to healthy control volunteers, providing clinical relevance to the role that AnxA1 might play in autoimmune diseases. We hypothesize that it may be possible to down-regulate the activity of autoreactive T and B cells from lupus patients by treating them with a neutralizing monoclonal antibody against the AnxA1.

The immunomodulatory activity of monoclonal anti-AnxA1 antibody was tested on an in vivo experimental model of human SLE. Six to eight week-old immunodeficient SCID mice were reconstituted by intraperitoneal (i.p.) injection of PBMCs from clinically relevant SLE patients. The control group of SCID mice was injected intraperitoneally with PBMCs from healthy volunteers. The humanized mice have been treated at weekly intervals with the monoclonal anti-AnxA1 antibody. All mice were bled weekly and collected sera were kept at -70ºC.

The changes of human cell populations after transfer in SCID mice with or without treatment with anti-AnxA1 antibodies were analyzed by flow cytometry (CD3, CD4, CD8, CD19, CD25, CD69). The kinetics of anti-DNA human IgG antibodies as well as the other auto-antibody panels and the cytokine secretion (IL-4, IL10 and IFN-γ) were determined by ultrasensitive ELISA. The glomerular deposition of human IgG were studied in selected animals. The degree of albumiuria was regularly measured. The life-span of the treated mice was prolonged compared to that of the controls.
Bilateral grant between Bulgarian Academy of Sciences and Hungarian Academy of Sciences

Current projects

ANTIBODIES WITH INDUCED POLYREACTIVITY – ROLE IN IMMUNE HOMEOSTASIS AND THERAPEUTIC POTENTIAL

Project leader: Tch. Vassilev, DSc

Research staff: A. Pashov, PhD; I. Djoumerska-Alexieva, PhD; M. Hadzhieva, PhD student; J. Dimitrov, PhD.

Antibody repertoires of healthy humans and animals contain a fraction of antibodies able to acquire additional polyspecificity following exposure to several biologically relevant redox molecules (free heme, reactive oxygen species (ROS), ferrous ions, HOCl, etc.). The physiological role of these "hidden" polyspecific antibodies is poorly understood. Similarly to inherently polyspecific antibodies, those with induced polyspecificity may also have immunoregulatory properties. We have previously shown that a pooled human IgG preparation, modified by the exposure of ferrous ions, acquires the ability to significantly improve survival of animals with polymicrobial sepsis or aseptic systemic inflammation induced by bacterial lipopolysaccharide or zymosan administration. In the present study we have analysed the effects of administration of heme-exposed pooled human IgG in the same models of sepsis and aseptic systemic inflammation. The administration of a single dose of heme-exposed pooled IgG resulted in a significant increase in the survival of mice with endotoxinemia, but not in those with polymicrobial sepsis and zymosan-induced severe generalized inflammation. Antibodies with induced polyspecificity acquire novel biological properties and therapeutic potential, however these depend on the mechanisms for modification used.

Grant DFNI-B01-29, National Science Fund

References:
DESIGN OF MODIFIED IMMUNOGLOBUIN PREPARATIONS WITH ADDITIONALLY INDUCED POLYSPECIFICITY FOR PASSIVE IMMUNOTHERAPY OF SEPSIS (BULGARIAN/SWISS COLLABORATIVE PROJECT)

Project leader: Tch. Vassilev, MD, DSc

Project collaborators: S. von Gunten, MD, PhD; C. Schneider, PhD student, University of Bern, Bern, Switzerland

Research staff: M. Hadzhieva, PhD student; A. Pashov, PhD; I. Djoumerska-Alexieva, PhD; J. Dimitrov, PhD; N. Bovin, PhD; S. von Gunten, PhD

On the basis of preliminary data, we have hypothesized that the passive immunotherapy with pooled immunoglobulin preparations with additionally enhanced polyspecificity could neutralize some of the products of the genomic storm and thus should be beneficial in systemic inflammatory syndromes, regardless of their primary insult. Three models of systemic inflammation in the presence or absence of infection were used to check this hypothesis: induced by LPS, induced by zymosan and induced by cecal ligation and puncture (CLP). Although the infusion of native IVIg had no effect on survival, the administration of the same single dose of the Fe(II)-exposed IVIg significantly improved the survival of mice in all three models. The studies of the mechanisms of beneficial action of the latter preparation revealed its ability to bind to proinflammatory molecules, complement components and extracellular histones.

Grant by the Swiss National Science fund and the Bulgarian Ministry of Education and Science (IZEBZO)

References:

STUDIES OF IMMUNOSIGNATURES IN THE ANTIBODY REPERTOIRES OF PATIENTS WITH PRIMARY MALIGNANT AND METASTATIC BRAIN TUMORS

Project leader: A. Bussarski, PhD

Project collaborator: Neurosurgery Clinic of the St. Ivan Rilsky Hospital of the Medical University in Sofia, Bulgaria

Research Staff: A. D. Pashov, PhD; D. Ferdinandov, MD; V. Kostov, student

The laboratory partnered in this project funded by an intramural microgrant from the Medical University. This collaborative project aimed at the building of a collection of sera from patients with glioblastoma multiforme, lung cancer brain metastases or non-tumor bearing surgery patients to probe the capacity of serum IgM reactivity to predict the diagnoses based on the patterns of binding to a large set of peptides from tumor antigens. The grant also covered the preliminary test with 3 tumor antigen microarray chips produced by the PepperPrint, Heidelberg.

Grant from the Medical University. The collection of sera is available and the analysis of 21 patients was further funded
RATIONAL DESIGN OF ANTIBODY REPERTOIRE PROBES USING PEPTIDE ARRAYS

Project leader: A. D. Pashov, PhD
Project collaborator: E. Hovig, PhD; T. Clancy, PhD, Oslo University Hospital, Oslo, Norway
Research Staff: Tch. Vassilev, DSc; M. Hadzhieva, PhD student, V. Shivarov, postdoc; V. Kostov, student

During the first year of the project data on the IgM reactivity of the sera from 21 patients with glioblastoma multiforme, lung cancer brain metastases or non-tumor bearing surgery patients was analyzed to determine the capacity of the IgM antibody repertoire dynamics to predict the diagnoses based on the patterns of binding to a large set of peptides from tumor antigens. 203 peptide reactivities differed significantly between the groups and could be used to classify the patients correctly ("leave on out" validation – 100%). Most of the work during this period was dedicated to adopting the appropriate bioinformatics methodology for the analysis of peptide array probes of antibody reactivity. A number of tools were designed and assembled together in a pipeline for the automation of the analysis.

In the framework of the BG09 D03-103 grant the Laboratory organized an international conference “The Antibody Repertoire as a Biomarker”, BAS, Sofia, 23.09.2015 (https://www.facebook.com/EAEconferenceARB/?ref=aymt_homepage_panel) with the participation of vaccinologists, immunologists and bioinformaticians from USA, France, Switzerland, Norway and Russia.

STUDY ON THE IMMUNE MODULATION OF DISEASE SEVERITY IN HUMANIZED SCID MURINE MODEL OF IMMUNOLOGICAL DISEASES

Project leader: A. Tchorbanov, PhD
Project collaborator: B.-L. Chiang, PhD; K.-H. Chu, PhD; C.-J. Chiu, PhD student; Graduate Institute of Immunology, National Taiwan University, Taiwan
Research staff: N. Mihaylova, PhD; K. Nikolova-Ganeva, PhD; I. Manoylov, PhD student

Systemic lupus erythematosus is a polygenic pathological disorder with multiple organ involvement. Self-specific B cells play a main role in the lupus pathogenesis by generation of autoantibodies as well as by serving as important autoantigen-presenting cells. Autoreactive T lymphocytes, on the other hand, are responsible for B cell activation and proliferation, and cytokine production. Therefore, both evidences promote the idea that a down-modulation of activated self-reactive T and B cells involved in the pathogenic immune response is a reasonable approach for SLE therapy.

Allergic diseases have been increasing in both developed and developing countries in the past decades. Allergic diseases such as bronchial asthma, allergic rhinitis and atopic dermatitis have been related to T helper cells function. Certain cytokines such as IL-4, IL-5, IL-13 have been found to play the critical
role in the pathogenesis of allergic diseases. For the treatment of allergic diseases, conventional therapeutic approaches including anti-histamine and steroid, novel treatments are still needed for these allergic diseases.

Most recently, we identified a particular subset of regulatory T cells induced by B cells (Treg/B cells), which could be applied for the future treatment of autoimmune or allergic diseases. In this collaborative project, we aim to study the role of regulatory T cells in the pathogenesis of both autoimmune and allergic diseases. In addition, we also like to explore the possibility on application of Treg cells, especially Treg/B cells for the treatment of immunological diseases.

Characterization of regulatory T cells induced by B cells for the treatment of autoimmune diseases. B cells isolated from Peyer’s patches and spleens were cultured with OVA and CD4⁺ CD25⁻ T cells. After 3 days, B cell-primed T cells (Treg-of-B cells) were harvested. The cytokine profile and suppressive function were assayed to characterize these Treg-of-B cells.

Bilateral grant between Bulgarian Academy of Sciences and Ministry of Science and Technology, Taiwan.
Final Summary:

STUDY OF THE PHYSIOLOGICAL, BIOCHEMICAL AND ECOLOGICAL CHARACTERISTICS OF MICROMYCETES RESISTANT TO HEAVY METAL STRESS

Project leader: M. Angelova, DSc
Project collaborator: V. Grishko, PhD, Ukrainian Academy of Sciences, Botanical Garden
Research staff: S. Pashova, MS; R. Abrashev, PhD; E. Krumova, PhD; N. Kostadinova, PhD; J. Miteva-Staleva, MS; B. Spassova, MS; E. Evtova.

Contamination of the environment by heavy metals is one of today’s serious problems. A number of industrial and agricultural activities cause a daily increase in the concentration of toxic waste in the soil and water. At the same time, heavy metals are associated with damages to human health. The aim of the project was to investigate the relationship between oxidative stress and heavy metal exposure in resistant fungal strains and the role of antioxidant defence in this resistance. The experiments were performed with two fungal strains Aspergillus fumigatus, isolated from contaminated soils in Bulgaria and Ukraine. A study on the adaptive response against copper, zinc, nickel and cadmium mixture as a admissible concentration limit (ACL) 0.75, 1.0, 2.0, 5.0 was carried out. The heavy metal effect was evaluated by the changes in fungal growth and metal ion accumulation, the level of oxidative stress biomarkers, and the antioxidant activities of enzymes such as superoxide dismutase (SOD) and catalase (CAT). The strain isolated from Ukraine has a higher threshold for the accumulation of metal ions in the cells. The adaptive response involved clearly pronounced oxidative stress events. The model strains demonstrated increase in oxidative damaged proteins content and reserve carbohydrates level. In addition, activation of antioxidant enzyme defence was evaluated. The tolerance to different concentrations of metal ions seems to be strain-dependent. Both strains, Aspergillus fumigatus 32 and Aspergillus fumigatus G exhibit varying degrees of damage from oxidative stress and different strategy of antioxidant protection. More clear response against metal-induced stress has been shown at the strain isolated from Bulgarian contaminated soil.

Bilateral joint project (2013-2015) in the frame of agreement between Bulgarian Academy of Sciences and National Academy of Sciences of Ukraine.

References:
EFFICACY OF ANTIMICROBIAL SUBSTANCES PRODUCED BY *STREPTOMYCES* IN MODIFYING POLYMERS USED IN CONSERVATION OF PAINTINGS IN SOME ANCIENT EGYPTIAN TOMBS

*Project leader:* M. Angelova, DSc

*Project collaborator:* M. Farrag, PhD, Zagazig University, Faculty of Science, Botany Department, Egypt

*Research staff:* S. Pashova, MS; R. Abrashev, PhD; E. Krumova, PhD; N. Kostadinova, PhD; J. Miteva-Staleva, MS; B. Spassova, MS; E. Evtova

Ancient Egypt is known for its invaluable culture monuments. Egyptian pyramids and tombs are a part of the world heritage. At the same time, they are exposed to various factors (physical, chemical and biological) that cause deterioration and degradation. Mural paintings and stone surfaces in the tombs suffer from different deterioration symptoms such as discoloration, structure alterations, and microbial penetration into painting layers. Filamentous fungi play an important role in biodeterioration of these objects.

The aim of the present project was to describe the fungal contamination in ten Egypt tombs and mosques as a first step in development of appropriate conservation method. For this purpose, molecular identification of the isolated fungal strains was performed. Also, the relation between composition of limestone support and fungal growth was proven.

The study provides new data of the harmful inhabitants of the Egypt tombs. A total of 30 isolates were obtained from the analyses of 13 samples taken from different tombs in Egypt through soil dilution and soil sprinkle plates techniques. All the strains were isolated from agar plates incubated at 28°C. The isolates were obtained in pure cultures by single conidial transfer onto beer agar plates. They were grouped and defined by classical taxonomy methods as filamentous fungi belonging to the phyla *Ascomycota* and *Basidiomycota*. Representatives of each morphology group and from each sample were successfully identified to species or genus level by sequencing analysis of ITS regions and the SSU ribosomal RNA gene. The results confirmed the affiliation of all isolates to the subkingdom *Dikarya*. Ten fungal strains belonged to *Penicillium* genus, four strains to *Aspergillus* genus, one strain to *Cladosporium* genus and one strain to *Bjerkandera adusta* species. Sequence comparison with the GenBank data (NCBI) showed over than 99 % identity with sequences from type strains or culture collection strains.

Bilateral scientific cooperation of the Bulgarian Academy of Sciences and the Zagazig University, Egypt.

*References:*
Current projects

**FUNGI AS SOURCE OF LIGNOLYTIC ENZYMES. PRODUCTION AND POSSIBLE APPLICATIONS.**

*Project leader:* E. Krumova, PhD,

*Project collaborator:* E. Savino, Department of Ecology and Land and Terrestrial Environments, University of Pavia, Italy

*Research staff:* M. Angelova, DSc; R. Abrashev, PhD; V. Dishliiska, MS; J. Miteva-Staleva, MS; N. Kostadinova, MS; S. Vassilev, MS; B. Spassova, MS; E. Evtova.

The study of wood decay macrofungi that naturally biodegrade lignocellulosic polymers has been steadily increasing due to their numerous and innovative applications. In the last decade, the researchers of Mycological Laboratory of Pavia University (Italy) and of The Staphan Angeloff Institute of Microbiology, BAS isolated in pure culture wood decay macrofungi collected in their own country. A few species have been investigated as regards different aspects: the growth profile at different temperature and the capacity to secrete cell wall degrading enzymes growing on poplar wood chips and sawdust. We tested four lignicolous species (*Daedalea quercina, Fistulina hepatica, Lenzites warnieri, Schizophyllum commune*) as regards their antioxidant activity. The two species poorly or not investigated till now for this activity, *D. quercina* and *L. warnieri*, revealed the most interesting results.

Italian-Bulgarian bilateral project (2014-2018)
LIST OF PUBLICATIONS NOT INCLUDED IN THE PROJECTS


Textbooks:


Kambourova, M., Toksoy Oner, E., Poli, A. Exopolysaccharides from prokaryotic microorganisms - promising sources for white biotechnology processes. In: Industrial Biorefineries and White Biotechnology (Pandey, A., Höfer, R., Taherzadeh, M.,


EDUCATION ACTIVITY

Lectures and Practical Exercise

Acad. A.S. Galabov, DSc
Course in Medical Virology, Kliment Ohridski University of Sofia, Faculty of Biology
Course in Antiviral Therapy, Kliment Ohridski University of Sofia, Faculty of Biology
Course in General Virology, New Bulgarian University

Corr. Member H. Najdenski, DSc
Course in Infectious Diseases, University of Forestry, Faculty of Veterinary Medicine, Sofia
Practical exercises in Infectious Diseases, University of Forestry, Faculty of Veterinary Medicine, Sofia
Course in Molecular Methods in Microbiology, New Bulgarian University
Practical exercises in Molecular Methods in Microbiology, New Bulgarian University
Course in Nutrition and Dietetic, New Bulgarian University
Practical exercises in Nutrition and Dietetic, New Bulgarian University

Corr. Member A. Pavlov, DSc
Course in Food Chemistry (for BS students), University of Food Technologies, Plovdiv
Practical exercises in Food Chemistry (for BS students), University of Food Technologies, Plovdiv
Course in Bioactive Substances from Cell Cultures (for MS students), University of Food Technologies, Plovdiv
Practical exercises in Bioactive Substances from Cell Cultures (for MS students), University of Food Technologies, Plovdiv
Course in Biotechnological Productions based on Plant and Cell Cultures (for BS students), Agricultural University, Plovdiv
Practical exercises in Biotechnological Productions based on Plant and Cell Cultures (for BS students), Agricultural University, Plovdiv
Course in Modern Technologies and Methods for Analysis of Bioactive Substances Chemistry (for BS students), University of Food Technologies, Plovdiv
Practical exercises in Modern Technologies and Methods for Analysis of Bioactive Substances Chemistry (for BS students), University of Food Technologies, Plovdiv

Prof. M. Angelova, DSc
Course in Oxidative Stress (for BS students), New Bulgarian University
Course in Mycology (for BS students), New Bulgarian University
Course in Mycology (for BS students), Plovdiv University Paisii Hilendarski.

Assoc. Prof. L. Doumanova, PhD
Course in Immunochemistry (for MS students), St Kliment Ohridski University of Sofia, Faculty of Chemistry
Practical exercises in Immunochemistry (for MS students), St Kliment Ohridski University of Sofia, Faculty of Chemistry
Assoc. Prof. S. Danova, PhD
Course in *The Probiotics* (for MS students), University of Chemical Technology and Metalurgy, Sofia, Department of Industrial Biotechnology

Assoc. Prof. S. Stoitsova, PhD
Course in *Methods of Electron Microscopy, Histochemistry and Immunocytochemistry in Cell Biology*, St. Kliment Ohridski University of Sofia, Faculty of Biology
Practical exercises in *Methods of Electron Microscopy, Histochemistry and Immunocytochemistry in Cell Biology*, St. Kliment Ohridski University of Sofia, Faculty of Biology

Assoc. Prof. Andrey Tchorbanov, PhD
Course in *Immunology* (for BS students), New Bulgarian University
Practical exercises in *Immunology* (for BS students), New Bulgarian University
Course in *Animal Models for Immunology of Reproduction*, Project for young investigators and PhD students, №BG051PO001-3.3.06-0059, Institute IBIR, BAS

Assoc. Prof. D. Beshkova, PhD
Course in *Microbial Safety of Animal Products*, (for MS students), University of Food Technologies, Plovdiv
Course in *Microbiology of Fermented Functional Foods* (for MS students), University of Food Technologies, Plovdiv

Assoc. Prof. E. Krumova, PhD
Practical exercises in *Oxidative Stress* (for BS students), New Bulgarian University
Practical exercises in Mycology (for BS students), New Bulgarian University
Practical exercises in *Cytology, Histology and Embryology* St. Kliment Ohridski University of Sofia, Faculty of Biology

Assoc. Prof. M. Mileva, PhD
Course in *Pharmacology of Eye Diseases* (for MS students), St. Kliment Ohridski University of Sofia, Faculty of Physics
Practical exercises in *Pharmacology of Eye Diseases* (for MS students), St. Kliment Ohridski University of Sofia, Faculty of Physic
Course in *Analytical Chemistry and Analysis of Medicinal Products*, Medical College "Yordanka Filaretova", Sofia
Practical exercises in *Analytical Chemistry and Analysis of Medicinal Product*, Medical College "Yordanka Filaretova", Sofia

Assis. Prof. V. Nicolova, PhD
Practical exercises in *Virology*, New Bulgarian University

Assist. Prof. S. Rusinova-Videva, PhD
Practical exercise in *Mycology* (for BS students), Plovdiv University Paisii Hilendarski, Plovdiv
Practical exercise in *Hydrobiology* (for BS students), Plovdiv University Paisii Hilendarski, Plovdiv
Practical exercise in *Hydrobiology* (for BS students), Plovdiv University Paisii Hilendarski, Branch Smolyan
Assis. Prof. N. Kostadinova, PhD
Practical exercises in Cytology and Cell Biology, St. Kliment Ohridski University of Sofia, Faculty of Biology

Assist. Prof. A. Marchev, PhD
Practical exercises in Biotechnological Productions Based on Plant and Cell Cultures (for BS students), Agricultural University, Plovdiv

Assist. Prof. R. Tropcheva, PhD
Practical exercises on Secondary Metabolites Biotechnology, St Kliment Ohridski University of Sofia, Faculty of Biology

Assis. Prof. A. Kroumov, PhD
Course in Photobioreactors-modeling, optimization, design and scale up, West Parana State University, Toledo, Parana, Brazil

Defended DSc Thesis

Danova S. Biodiversity and probiotic potential of lactic acid bacteria from different ecological niches

Defended PhD Thesis

Gesheva V. Modulation of immune response by hemocyanins from Rapana thomasiana
Advisor: Assoc. Prof. A. Tcorbanov, PhD and Assoc. Prof. K. Idakieva, PhD

Hubenov, V. Studies of anaerobic digestion of organic wastes at mesophilic and thermophilic conditions”
Advisors: Assoc. Prof. I. Simeonov, PhD and Assoc. Prof. D. Denchev, PhD

Kerekov N. Humanized experimental models of autoimmune and allergic diseases.
Advisor: Assoc. Prof. A. Tcorbanov, PhD

Milanova V. Inflammatory and destructive potential of neutrophiles in arthritis.
Advisor: Assoc. Prof. P. Dimitrova, PhD

Pashova S. Applied investigations of B cell populations.
Advisor: Assoc. Prof. A. Pashov, PhD

PhD students

Belenksa L. Role of complement in the processes of joint destruction in experimental models of arthritis.
Advisor: Prof. N. Ivanovska, DSc

Dimitrova L. New antimicrobial agents with therapeutic potential

Ganchev I. Mixed biofilms.
Advisor: Assoc. Prof. S. Stoitsova, PhD
Ganova P. *Influence of tyrosine kinase inhibitors on the processes of osteoclastogenesis in experimental arthritis.*
Advisor: Prof. N. Ivanovska, DSc

Gotova I. *Study of probiotic characteristics of lactic acid bacteria and selection of strains for products with health benefits*
Advisors: Corr. Member H. Najdenski, DSC and Assoc. Prof. J. Dimitrov, PhD

Hadjieva M. *Induced polyspecificity of antibodies.*
Advisor: Prof. T. Vassilev, MD, DSc

Hinterholz C. L. *Cultivation of algae in a new photo-bioreactor for the sequestration of carbon dioxide from waste gases of various types and synthesis of high value products.* West Parana State University, Toledo PR, Brazil.
Advisor: Assis. Prof. A. Kroumov, PhD

Ivanova I. *Antigen targeting by genetically engineered chimeric molecules*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Litova K. *Microbial biodegradation of industrial pollutants of the environment*
Advisor: Assoc. Prof. Z. Alexieva, PhD

Manoilov I. *Specific therapy of autoimmune diabetes in mouse and humanized models.*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Miteva-Staleva J. *Cold stress and aging in Antarctic fungi*
Advisor: Prof. M. Angelova, DSc

Nemska V. *Biotechnological and functional characteristics of probiotic lactic acid bacteria*
Advisor: Assoc. Prof. Svetla Danova, DSc

Stoyanov A. *Genetic and molecular studies on methylotrophic yeast Hansenula polymorpha*
Advisor: Assoc. Prof. K. Lahtchev, PhD

Stoykov Y. *Production and characterization of microbial chitinases*
Advisors: Prof. A. Pavlov, DSc and Prof. A. Krastanov, DSc

Teneva Ts. *Biodiversity of lactic acid bacteria in Bulgarian medical plants and solution for using their biological potential*
Advisor: Assoc. Prof. D. Beshkova, PhD

Velikova P. *Polysaccharide-modifying lactic acid bacteria with application in the food industry*
Advisor: Assoc. Prof. P. Petrova, PhD

Vancheva R. *Bioactive substances from Bulgarian Fumaria spp.*
Advisor: Prof. A. Pavlov, DSc

Zahmanov G. *Pharmacetically important metabolites from from Sambucus ebulus and its in vitro cultures*
Advisor: Assoc. Prof. M. Georgiev, PhD

**Defended MS Diploma Thesis**

Adamov A. *Biosorption potential of biomass of Antarctic strain Cryptococcus laurentii AL65.*
Advisor: Assist. Prof. S. Rusinova-Videva, PhD

Andrejnski N. *Cell response of two strains Aspergillus fumigatus*, isolated from heavy metal polluted regions against metal-induced toxicity
Advisor: Assoc. Prof. E. Krumova, PhD
Bratanova D. *Study on Lactobacillus microbiota of breast milk*
Advisor: Assoc. Prof. Svetla Danova, DSc

Boteva N. *Studies at newly isolated Lactobacilli from fermented dairy products*
Advisor: Assoc. Prof. Svetla Danova, DSc

**Defended BS Diploma Thesis**

Delcheva N. *Suppression of auto-reactive B and T lymphocytes from diabetis patients by protein chimeric molecules.*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Dyankova D. *Antioxidant enzyme defence of filamentous fungi isolated from heavy metal polluted soils*
Advisor: Assoc. Prof. E. Krumova, PhD

Kolev K. *Selective elimination of specific B-lymphocytes by protein-engineered chimeric molecules.*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Petkova N. *Production and characterization of the antioxidants enzyme superoxide dismutase from the strain Aspergillus fumigatus 32, resistant to heavy metals*
Advisor: Assist. Prof. R. Abrashev, PhD

Slavov S. *Functional elimination of auto-reactive T cells in murine MRL/lpr model of lupus by therapy with antibody against Annexin A1.*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

**Postdoc practice**

Semcheddine S. *Maitre de conference*
Advisor: Assoc. Prof. Ivan Simeonov, PhD

**National Student Practice financed by Operational Programme “Human Resources Development”, co-financed jointly by the European Social Fund of the EU**

Bradyanova S. *Experimental immunology*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Chepinski P. *Experimental immunology*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Delcheva N. *Experimental immunology*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Hlebarska D. *Experimental immunology*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Slavov S., *Experimental immunology*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Valentinov K., *Experimental immunology*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Boneva G., *Experimental immunology*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Kostadinova V., *Experimental immunology*
Advisor: Assoc. Prof. A. Tchorbanov, PhD
CELEBRATION DAY OF THE STEPHAN ANGELOFF INSTITUTE OF MICROBIOLOGY

The Day of Institute, March 14, already turned into tradition, was celebrated with a ceremonial meeting. The meeting was opened with exciting speech delivered by the Director - Prof. H. Najdenski, Corresponding Member of BAS. Special guest and invited speaker in 2015 was Prof. Lydmila Boyanova, MD, DSc, from Medical University of Sofia that gave a very intriguing lecture entitled “Helicobacter pylori – the carcinogenic bacteria”.

Acad. Angel S. Galabov, delivered the traditional Annual Prize for the best scientific publication by young microbiologist (under 35 year of age) to Tsvetelina Paunova-Krusteva, PhD (The Stephan Angeloff Institute of Microbiology, Department of General Microbiology).

ACTIVITIES OF FOUNDATION “ACAD. PROF. DR. STEPHAN ANGELOFF”

The annual awards for the best scientific publication by young microbiologist (under 35 year of age) were been given to Tsvetelina Paunova-Krusteva, PhD (The Stephan Angeloff Institute of Microbiology, Department of General Microbiology) for the following paper:


SCIENTIFIC SYMPOSIUM „BENEFICIAL AND PATHOGENIC MICROBES FOR HEALTHIER LIFE AND SAFETY FOODS”. Chairpersons: Assoc. Prof. SVETLA DANOVA, DSc and Corr. Member HRISTO NAJDENSKI, DSc

The Stephan Angeloff Institute of Microbiology (Bulgarian Academy of Sciences) in cooperation with the United Scientific Group (USG) hosted the scientific symposium Beneficial and pathogenic microbes for healthier life and safety foods on April 8-9, 2015 at Institute of Microbiology, Acad. G. Bontchev, str. 26, 1113 Sofia, Bulgaria. The two days scientific symposium, was a pre-conference event of the Probiotic summit 2015 San Francisco, USA (http://www.unitedscientificgroup.com/conferences/probiotics-summit/)

The symposium explored critical points in application of beneficial microbes – probiotics and the risk of food-borne pathogens:

- for safety and functional food;
- new approaches for prevention the microbial risks in food chain from pathogens;
- new trends in protection of human and animals health;
- prophylaxis and therapy of food disorders for wellbeing and health.

The symposium provided an opportunity to the participating scientists and young researchers to discuss various issues pertaining to their research career. It gived an understanding of the beneficial and pathogenic microbes, functional and safety foods for long and healthier life.

The symposium assembled scientists from different Universities/Institutions (Bulgarian Academy of Sciences, Sofia University “St. Kliment Ohridski”, Plovdiv University “Paisij Hilendarski”, Bulgarian Food Safety Agency, Center for Risk Assessment, etc.) that have developed innovative researches and analyses to these problems and discussions by education leaders about applications and solutions for other activities that could relieve critical situation and improve the know-how and efficiency of the coming generation.

Site of the event: www.microbio.bas.bg/conferences/probiotics
NATIONAL CONFERENCE WITH INTERNATIONAL PARTICIPATION “ECOLOGICAL ENGINEERING AND ENVIRONMENT PROTECTION”

Fourth National Conference with International Participation and Youth Scientific Session „Ecological Engineering and Environmental Protection” EEEP was held on 03-06 June 2015, at Burgas, Bulgaria.

INTERNATIONAL IMMUNOLOGY SCHOOL, Organizer: Assoc. Prof. ANDREJ TCHORBANOV, PhD - President of Bulgarian Society for Immunology (BuSI)

2nd Black sea International Immunology school (BSIIS2015) was held on 15-16 October 2015 in Hotel “Legends” - Sofia, Bulgaria. The scool was funded by EFIS.

AWARDS FOR 2015

Award “Pitagor”
Assoc. Prof. Milen Georgiev, PhD (Department of Applied Microbiology) got the Winner in the "Pythagoras" award for an established scientist in the technical sciences.

Awards of the Union of Scientists in Bulgaria - 2015
Assoc. Prof. Petya Dimitrova, PhD (Department of Immunology) received a Diploma of the Bulgarian Union of Scientists for Excellence thesis of her PhD student Victoria Milanova.
Assist. Prof. Kalina Nikolova-Ganeva, PhD (Department of Immunology) was awarded a price of the Bulgarian Union of Scientists for 2015 year in the field of Biology and Medicine.
Assist. Prof. Tsvetelina Paunova-Krusteva, PhD (Department of General Microbiology) was awarded a price of the Bulgarian Union of Scientists for excellence in PhD thesis for 2015.

Award of Evrika Foundation
Assoc. Prof. Petya Dimitrova, PhD (Department of Immunology) was awared a price of Evrika Foundation for for Excellence thesis of her PhD student Victoria Milanova

Awards of Congress and Conferences
Assoc. Prof. Petya Dimitrova, PhD (Department of Immunology) received the TEWA award for the best oral presentation on 2nd ICNPU, Plovdiv, Bulgaria.
Assist. Prof. Maya Zaharieva, PhD (Department of Infectious Microbiology) received the TEWA award for the best poster on 2nd ICNPU, Plovdiv, Bulgaria.
Staff of the Stephan Angeloff Institute of Microbiology

SCIENTIFIC DIVISION

DEPARTMENT OF GENERAL MICROBIOLOGY

Head of Department: Zlatka Aleksiieva, PhD, Assoc. Professor

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Lidana Panova, BS, Laboratory Assistant
Ivo Ganchev, PhD student

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DEPARTMENT OF APPLIED MICROBIOLOGY

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Laboratory of Microbial Biosynthesis and Ecology

Head: Kolishka Tcekova, PhD, Assoc. Professor

Adriana Gousterova, PhD, Assoc. Professor
Lyudmila Kabaivanova, PhD, Assoc. Professor
Aleksander Krumov, PhD, Assist. Professor
Dessislava Todorova, PhD, Assist. Professor
Ignat Dimov, Assist. Professor
Lilyana Nacheva, Assist. Professor
Philip Philipov, Researcher
Rositca Petkova, Researcher
Anna Brachkova, PhD student

Laboratory of Extremophilic Bacteria

Head: Margarita Kambourova, DSc, Assoc. Professor

Ivanka Bojadjzheva, PhD, Assist. Professor
Miroslava Atanasova, PhD, Assist. Professor
Iva Tomova, PhD, Assist. Professor
Nadja Kirilova, PhD, Assist. Professor
Nikolina Atanasova, MS, Assist. Professor

Laboratory of Applied Biotechnologies

Head: Atanas Pavlov, DSc, Professor

Milen Georgiev, PhD, Assoc. Professor
Kostanac Pavlovska, PhD, Assoc. Professor
Dora Beshkova, PhD, Assoc. Professor
Ginka Frengova, PhD, Assoc. Professor
Vasil Georgiev, PhD, Assist. Professor
Snejana Videva, PhD, Assist. Professor
Andrei Marchev, PhD Assist. Professor  
Katerina Georgieva, MS, Microbiologist  
Tatiana Gocheva, Technologist  
Elka Genova, Technologist  
Svetla Stojkova, Technologist  
Vasilka Ushterova, Laboratory Assistant  

Tzvetanka Teneva, PhD student  
Yuri Stoikov, PhD student  
Georgi Zahmanov, PhD student  

Research group of Mathematical modelling and Computer Methods  
Head: Ivan Simeonov, PhD, Assoc. Professor  
Elena Chorukova, PhD, Assist. Professor  
Venelin Hubenov, Assist. Professor  
Snezhanka Mihaylova, MS, Technologist  
Georgi Valevski, Dipl. eng.  

DEPARTMENT OF INFECTIOUS MICROBIOLOGY  
Head of Department: Hristo Najdenski, DVM, DSc, Professor, Corr. Member of BAS  
Laboratory of Zoonoses and Bacterial Virulence  
Head: Hristo Najdenski, DVM, DSc, Professor, Corr. Member of BAS  
Maya Zaharieva, PhD, Assist. Professor  
Violeta Valcheva, PhD, Assist. Professor  
Zvezdima Tcvetanova, PhD, Assist. Professor  
Magdalena Bonovska, PhD, Researcher  
Trayana Draganova, MS, Researcher  
Dimitar Dimitrov, Assist. Professor  
Vanja Slaveva, Researcher  
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Laboratory of Genetics and Resistance of Mycobacteria  
Head: Nadya Markova, MD, PhD, Assoc. Professor  
Georgi Slavchev, MS, PhD student  
Albena Cherneva, Researcher  

Laboratory of Antimicrobial Agents  
Head: Vesselin Kusovski, PhD, Assoc. Professor  
Iva Tzvetkova, MS, Researcher  

Tchavdar Tankov, Laboratory Assistant  

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Academician Angel S. Galabov, MD, DSc, Professor  

Laboratory of Viral Proteins  
Head: Lyubka Doumanova, PhD, Assoc. Professor  
Milka Mileva, PhD, Assoc. Professor  
Yurii Abashev, PhD, Assist. Professor  
Adriana Dimitrova, PhD student  

Laboratory of Experimental Chemotherapy of Enteroviral Infections  
Head: Ivanka Nikolova, PhD, Assist. Professor  
Nadya Nikolova, MS, Researcher  
Snejana Andreeva, Laboratory Assistant  

Laboratory of Experimental Chemotherapy of Influenza  
Head: Lora Simeonova, PhD, Assist. Professor  
Galina Gegova, MS, Researcher  
Kirilka Todorova, BS, Technician  

Laboratory of Oncolytic Viruses  
Head: Assya Angelova, PhD, Assist. Professor  
Zahari Raykov, MD, PhD, Assist. Professor  
Neli Vilhelmova, PhD, Assist. Professor  
Adelina Stoyanova, MS, Assist. Professor  
Luchia Mukova, MS, Researcher  

DEPARTMENT OF IMMUNOLOGY  
Head of Department: Nina Ivanovska, DSc, Professor  

Laboratory of Experimental Immunotherapy  
Head: Anastas Pashov, PhD, Assoc. Professor  
Tchavdar Vassilev, MD, DSc, Professor  
Petya Dimitrova, PhD, Assoc. Professor
Iglica Djoumerska-Alexieva, MD, PhD, Assist. Professor
Maya Hadjieva, PhD student

**Laboratory of Experimental Immunology**
**Head:** Andrey Tchorbanov, PhD, Assoc. Professor
Nikolina Mihaylova, PhD, Assist. Professor
Kalina Nikolova-Ganeva, PhD, Assist. Professor
Ilian Manoilov, PhD, Assist. Professor
Nikola Kerekov, PhD, Assist. Professor
Viktoriya Milanova, PhD student

**Laboratory of Infectious Immunology and Inflammation**
**Head:** Nina Ivanovska, DSc, Professor
Tsvetanka Stefanova, PhD, Assist. Professor
Valeriya Gyurkovska, PhD, Assist. Professor
Petya Stoyanova, DVM, Researcher
Iva Ivanova, PhD student

**ADMINISTRATIVE AND TECHNICAL DIVISION**

**Secretariat**
Petya Nikolova, Coordinator
Nadka Panova, Human Resources
Ivailo Georgiev, MS, IT Service eng
Krasya Nenova, Technical secretary
Ivan Georgiev, Secretary
Nora Toupareeva, Courier

**Accountancy**
Antoaneta Tcareva, Chief accountant
Stefka Yonkova, Accountant
Snezhanka Daskalova, Accountant-cachier
Violeta Manolova, Accountant

**Public Relations**
Violeta Valcheva, PhD, Assist. Professor
Lora Simeonova, PhD, Assist. Professor

**Library**
Todorka Kehajova, Librarian
Anna Vaneva, Researcher

**BIOLOGICAL SERVICES**

**Laboratory Cultural Media**
Nataliya Georgieva, MS, Researcher

Petya Ganova, PhD student

**SECTION OF MYCOLOGY**
**Head:** Ekaterina Krumova, PhD, Assoc. Professor
Maria Angelova, DSc, Professor
Radoslav Abrashev, PhD, Assist. Professor
Nedelina Kostadinova, PhD, Assist. Professor
Jeni Miteva-Staleva, MS, Assist. Professor
Vladislava Dishliiska, MS, Assist. Professor
Borjana Spasova, MS, Researcher
Emilia Eftova, Laboratory Assistant

**LABORATORY CENTER PASTEUR**
**Head:** Peter Grozdanov, PhD, Assist. Professor
Anna Terziyska, PhD, Assist. Professor

Dima Kuzarova, MS
Krasimira Beshkova, Technician

**Fermentation Laboratory**
Spasen Vassilev, Assist. Professor
Vesselina Pankova, MS, Researcher eng.
Galina Nikolova, MS

**Animal House Facility**
Petya Stoyanova, DVM, Researcher
Eleni Axioti Dimitrova
Ivanka Zachova

**Technical Service**
Evgenia Minkova, Assitant director, eng.
Electro-Mechanical Workshops
Dimiter Brajnov
Plamen Stefkov
Tsvetanka Begova
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<td>Danka Galabova</td>
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<td>Margarita Kamburova</td>
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<td>Stoyanka Stoitsova</td>
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<td>Nadya Markova</td>
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<td>Anna Erdei</td>
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**NATIONAL SCIENTIFIC SEMINARS**

**General Microbiology**

*Chairman:* Assoc. Prof. S. Danova, DSc  
*Vice Chairman:* Assoc. Prof. S. Stoitsova, PhD  
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**Pathogenic Microorganisms and Infectious Immunology**

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*Vice Chairman:* Assoc. Prof. L. Doumanova, PhD  
*Secretary:* Assist. Prof. V. Nikolova, PhD

*Carried out in the Stephan Angeloff Institute of Microbiology, BAS*
Press Conference of the SAIM-BAS (November 12, 2015) in Bulgarian News Agency (BTA): “Barriers to the best microbiological institute in South-East Europe”. The former Director Acad. Angel S. Galabov, current Director Corr. Member Hristo Najdenski and scientists from the Institute expressed their opinion about the problems concerning research funding.

The consecutive 9th Balkan Congress of Microbiology, Microbiologia Balkanica’2015, was carried out in Thessaloniki (Greece) at October 22-24, 2015. More than 230 is the number of registered participants, 53 of which young scientists. SAIM - BAS was presented by 28 scientists, giving 4 plenary lectures, 12 oral presentations and 15 posters.
Celebration “Day of the Stephan Angeloff Institute of Microbiology”, March 14, 2015. The Director - Prof. Hristo Najdenski, Corresponding Member of BAS congratulates Prof. Lydmila Boyanova, MD, DSc, from Medical University of Sofia presenting a very intriguing lecture entitled “Helicobacter pylori – the carcinogenic bacteria”.

The President of the Foundation “Acad. Prof. Dr. Stephan Angeloff”, Acad. Angel S. Galabov, delivers the traditional annual prize for the best scientific publication by young microbiologist to Tsvetelina Paunova-Krusteva, PhD from The Stephan Angeloff Institute of Microbiology, Department of General Microbiology.

Scientific Conference with international participation “The Antibody repertoire as a biomarker”, September 23, 2015, Sofia (BG09 PROGRAMME EEA SCHOLARSHIPS FUND). The chairman of the Organizing Committee is Assoc. Prof. Anastas Pashov, PhD.
2nd International conference on “Natural products utilization: from plants to pharmacy shelf”, October 14-17, 2015, Plovdiv, Bulgaria. The chairman of the Organizing Committee is Assoc. Prof. Milen Georgiev, PhD.
EDITORS
Professor H. Najdenski, DVM, DSc, Corr. Member of BAS
Professor M. Angelova, DSc

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