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The Stephan Angeloff Institute of Microbiology

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SCIENTIFIC REPORT 2016

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DEPARTMENT OF GENERAL MICROBIOLOGY

SECTION OF MORPHOLOGY OF MICROORGANISMS AND ELECTRON MICROSCOPY

Current project

INVASIVENESS AND BIOFOLM FORMATION ADAPTATIONS FOR PERSISTENCE OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM PATIENTS WITH CYSTIC FIBROSIS AFTER TOBRAMYCIN THERAPY

Principal investigator: T. Paunova-Krasteva

Supervisor: S. Stoitsova

Cystic fibrosis is a hereditary disease related with the most common lethal single gene disorder in Europe and is associated with organ inflammation and damage particularly affecting the lungs. Complications are due to bacterial infections among which *P. aeruginosa* is a leading cause of exacerbations and early death. The development of resistance to antibacterials creates serious problems to therapy, tobramycin remaining one of the still successful antibiotics, however its effects may be hindered by the development of persistence. The aim of the project is a comparative investigation of *P. aeruginosa* clinical strains isolated prior- and post-tobramycin therapy. We focused on bacterial phenotypes like motility, growth dynamics and biofilm formation by the strains. It was established that both early and post-treatment strains are sensitive to tobramycin. No specific differences between early and post-treatment isolates were established in swimming, swarming and twitching motility. A phenomenon worth mentioning is the distinct prolongation of the lag-phase of growth among post-treatment strains which could indicate an adaptation for persistence. The cystic fibrosis lung, due to the presence of significant amounts of sputum, is characterized by microhabitats where gradients of various substances including antibiotics may occur. For this reason we tested the effects of 1/4 and ½ MIC of tobramycin on bacterial growth as plankton or as biofilm. We showed that the sub-inhibitory amounts of tobramycin significantly suppressed growth in the liquid phase, but did not prevent the formation of significant amounts of biofilm by the isolates.

Contract No DFNP-55/27.04.2016, supported by "Programme for Carrier Development of Young Scientists", BAS

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Paunova-Krasteva Ts.,Velkova V., Borisova D., Stoitsova S. Intracellular survival of *Pseudomonas aeruginosa* PAO1 in

A549 cells. *Annuaire de l'Université de Sofia "St. Kliment Ohridski"Faculte de Biologie*. 2016, 102 (4), 170-175.

Borisova D., Strateva T., Paunova-Krasteva Ts., Mitov I., Stoitsova S. Phenotypic investigation of paired *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients prior- and post-tobramycin treatment. *C. R. Acad. Bulg. Sci.* 2016 (in press).

Paunova-Krasteva Ts., Borisova D., Strateva T., Mitov I., Stoitsova S. Effects of sub-MICs of tobramycin on growth and biofilm formation by clinical cystic fibrosis paired strains of *Pseudomonas aeruginosa* isolated prior and post-tobramycin treatment. In: *Antimicrobial resistance in microbial biofilms and options for treatment*, Ghent, Belgium, October 5-7, 2016, O59.

Paunova-Krasteva Ts.,Velkova V., BorisovaD.,Stoitsova S. Intracellular survival of *Pseudomonas aeruginosa* PAO1 in A549 cells.*Youth Scientific Conference "Kliment`s Days*", November 17-18, 2016, Sofia, Bulgaria, p. 74.

New Project

THE ROLE OF EXOY NUCLEOTIDYL CYCLASE TOXIN IN *PSEUDOMONAS AERUGINOSA* INFECTIONS

Project supervisor: Undine Mechold, PhD, Institute Pasteur, Paris

Project leader: S. Stoitsova, PhD

Research Staff: L. Touqui; Ts. Paunova-Krasteva, PhD; D. Borisova, Ms; A. Belyy, PhD; G. Karimova; D. Raoux-Barbot; D. Ladant; H. Silvestre; C. Abrial; J. Da Silva; L. Renault

Contract No PTR-43-16 with Institut Pasteur, Paris, France

SECTION OF MICROBIAL GENETICS

Current projects

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

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

Project leader: P. Petrova, PhD

Project collaborators: Institute of Chemical Engineering, BAS, Sofia

Research staff: P. Velikova, PhD; A. Stoyanov, 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 following genes were assayed for transcription products: *amy1*, encoding α-amylase, EC 3.2.1.1; *glgP* (glycogen-phosphorylase, EC 2.4.1.1); *glgB* (1-4-α-glucan branching enzyme, EC 2.4.1.18); *agl* (alpha-glucosidase, EC 3.2.1.20); *malL* (oligo-1,6-glucosidase, EC 3.2.1.10); *treC* (trehalose-6-phosphatehydrolase, oligo-1,6-glucosidase, EC 3.2.1.93); and *dexC* (neopullulanase, EC 3.2.1.33). 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.

Grant DFNI B02/27 of the Bugarian Science Fund, Ministry of Education and Science

References

Velikova P., Stoyanov A.**,** Blagoeva G., Popova L., Petrov K., Gotcheva V., Angelov A., Petrova P. Starch utilization routes in lactic acid bacteria: New insight by gene expression assay. Starch - Stärke, 68, 9-10, WILEY-VCH Verlag GmbH & Co, 2016, ISSN: 1521-379X,

DOI:10.1002/star.201600023, 953-960. SJR: 0.513.

Petrova P., Petrov K. Traditional Cereal Beverage Boza - Fermentation Technology, Microbial Content and Healthy Effects. In book: Fermented Food - Part II: Technological Interventions, Chapter: 13, Publisher: CRC PRESS, Boca Raton, FL, USA, Editors: Ramesh C. Ray and Didier Montet, pp. 284-305, ISBN 9781138637849 (in press).

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; Bright Dairy & Foods Co. Ltd, Shanghai, China

Research staff: P. Velikova, PhD; A. Stoyanov, PhD; R. Eneva, PhD; E. Kroumova, PhD; J. Miteva-Staleva, PhD; M. Zaharieva, PhD; K. Petrov, PhD; F. Tsvetanova, PhD

The specific natural and climatic conditions in Bulgaria had contributed for the spontaneous evolution of starter cultures, usually associated with the good health and the longevity of the yogurt consumers. Aiming to isolate unique strains of Lactic acid bacteria (LAB) that have acquired special properties over the centuries, about eighty distinct yogurt samples from remote territories (towns-museums, small villages, monasteries) in the country were collected.

Although there are several regions, known to contain endemic micro-flora (Tran, Stara planina, Tracian and Rose valleys), the largest biodiversity of LAB was observed in the samples from Rhodope Mountains. The strains were isolated from cow's, sheep's, goat's, or buffalo's yogurt, homemade according to the ancient national receipts. The new isolates belonged to the species *Lactobacillus bulgaricus, L. helveticus, L. rhamnosus, L. fermentum, L. paracasei*, and genera *Pediococcus, Streptococcus, Enterococcus, Leuconostoc*, and *Weissella*, according to the results obtained by 16S rDNA sequencing. The strains were characterized in terms of their acidifying ability (in skimmed milk, or in 4% Lactose medium); the percentage of the optical isomers of lactic acid; exopolysaccharides production; proteolytic activity and the capability to produce antimicrobial substances. Several *L. bulgaricus* strains were found to synthesize galacto olygosaccharides (GOS), which are known as valuable prebiotic carbohydrates.

The comprehensive genetic characterization of particular LAB strains and the further analysis of the liquid and volatile metabolites are in progress. These results would uncover the traits bringing the authentic Bulgarian yogurt fragrance and the potential beneficial effects. However, the known origin and the good technological properties of the new isolated strains are always enough to allow their successful application in starters for the "Original Bulgarian type yogurt" manufacturing.

Grant by Bright Dairy & Foods Co. Ltd, China.

New project

INVESTIGATION ON THE PROCESSESOF MINING WASTEWATER TREATMENTTHROUGH INTEGRATED MFCS

Project supervisor: A. Angelov, PhD, University of Mining and Geology "St. Ivan Rilski", Sofia

Project leader: Z. Alexieva, PhD

Project collaborators: University of Mining and Geology "St. Ivan Rilski", Sofia; Institute of Chemical Engineering, BAS, Sofia; University of Chemical Technology and Metallurgy (UCTM), Sofia

Project staff: M. Gerginova, PhD; N. Peneva, MS; K. Stoyanova, PhD student

SECTION OF MICROBIAL BIOCHEMISTRY

New Project

ROLE OF AMARYLLIDACEAE ALKALOIDS IN THE ENDOPHYTE-HOST RELATIONSHIPS

Project leader: S. Berkov, Instute of Biodiversity and Ecosystem Research, BAS

Project staff: B. Mutafova, PhD; A. Sotirova

Grant DN01/13 of the Bugarian Science Fund, Ministry of Education and Science

DEPARTMENT OF INFECTIOUS MICROBIOLOGY

LABORATORY OF ZOONOZES AND BACTERIAL VIRULENCE

Final summary

EVALUATION OF THE CHEMICAL STABILITY AND BIOLOGICAL ACTIVITY OF ANTIMICROBIAL PEPTIDES TYPE ALFA-DEFENSINE 2

Project supervisor: V. Pencheva, Faculty of Pharmacy, Medical University Sofia

Project leader: H. M. Najdenski, DSc,

Project collaborator: Faculty of Pharmacy, Medical University, Sofia

Research staff: M. M. Zaharieva, PhD.

During the last 20 years we are witnessing an increasing resistance among pathogenic bacteria against numerous clinically approved, often lifesaving antibiotics. Therefore, increasing attention is paid to specific active compounds such as antimicrobial peptides (AMP). They are composed of 12 to 50 amino acid residues and are amphipathic molecules with a broad antibacterial profile, both against Gram-positive and Gram-negative bacteria. Many authors consider them as the modern alternative to antimicrobial chemotherapy. Parallel to their advantages, however, there are a number of complications that impede their clinical application. One of the most important is deactivation by the pH of the medium. The main scientific idea and hypothesis of this project were the development of analytical procedures for assessing the chemical stability of alpha-defensin 2 in different model media with the addition of modulating factors and a parallel assessment of their biological activity against pathogenic bacteria from the species *Staphylococcus aureus* and *Escherichia coli*.

All tests for antimicrobial activity were performed based on ISO 20776/1-2006. The respiratory activity of the bacteria in treated samples *versus* untreated control was measured using a tetrazolic salt as reducing agent. Samples with decreased metabolic activity or with no visible growth were seeded on agar plates to determine the presence of bactericidal effect or the level of growth inhibition measured through the number of colony forming units.

Among the four buffers with different pH for dissolving of the alpha-defensin 2, only buffer with pH 9 showed moderate activity in difference to the other three buffers (pH 2, 4 and 7.4). The solution was active against the *E. coli* test-strain in concentration 10 μg/ml. The effect was bacteriostatic and the respiratory activity of the treated bacteria was diminished by 20% more than the negative buffer (pH 9.0) control.

In conclusion, the tested peptide exhibited moderate bacteriostatic effect against pathogenic *E. coli* bacteria and was not active against pathogenic *S. aureus* strains. Buffer with pH 9.0 provides higher protein stability than the other three buffers, but not long enough to fully deploy the bactericidal potential of the alpha-defensin 2 over time. A good strategy for increasing

the stability of the AMP would be the development of nanoparticles.

Grant 47/2016, Faculty of Pharmacy, Medical University, Sofia

References:

Americova M. N. MS thesis: Assessment of the chemical stability and biological activity of antimicrobial peptides type alfa-defensin 2, Faculty of Pharmacy, Medical University – Sofia (H. Najdenski, V. Pencheva, supervisors).

Current projects

SURVEILLANCE OF *YERSINIA ENTEROCOLITICA* IN SLAUGHTER PIGS BY USING LOW COST BUT EFFICIENT DNA AMPLIFICATION METHODS

Project leader: H. Najdenski, DSc

Project collaborators: Els van Collie, Mark Heyndricks, Lieve Herman, Unit "Food Safety", Institute for Agriculture and Fisheries Research(ILVO), Melle, Belgium

Research staff: M. Zaharieva, PhD; M. Gatzovska, MS; V.Teneva, MS

Since 2013 Yersiniosis has been the third most commonly reported zoonosis in the European Union. Slaughter pigs are considered one of the main transmission routes that could result in contaminated pork meat and a food-borne outbreak. The ISO based detection and enumeration method for pathogenic *Yersinia* spp. in meat samples remains a gold standard although it is a difficult and time-consuming. It is usually combined with conventional PCR end-poin tanalysis for confirmation of the detected species but it is characterized by a low sensitivity, whereas the quantitative TaqMan PCR remains still too expensive for routine applications and therefore not applicable in low performing EU Member States, such as Bulgaria. In this study, we applied previously optimized by our research group protocols and primer sets for a surveillance report of *Y. enterocolitica* in slaughter pig samples proving the advantages of the LAMP protocol for specific, sensitive and inexpensive detection of the pathogen.

All bacteriological procedures were performed according to BS EN ISO 10273-2003. From each tonsil or faeces, 0.2 g was frozen for direct DNA isolation. Bacterial lysates from suspected colonies were tested for the presence of the *ail* gene with conventional PCR protocol. Pure DNA obtained from the *ail*-positive isolates was subjected to LAMP assay for comparison and confirmation of the PCR result. Direct DNA isolation from the frozen samples was done with the EURxDNA Stool isolation kit. A primer set, complementary to the gene *phoP*[2,3], checked for selectivity and sensitivity in previous investigations (2015), was used for the LAMP reaction. The products of the LAMP reaction were visualized with the dye hydroxynaphthol blue and DNA electrophoresis in agarose gel. The surveillance of *Y. enterocolitica* in the tested pig tonsils was summarized based on the place of origin.

In total, 75 samples were processed by the ISO standard of which 11 were positive for the *ail* gene as shown by conventional PCR. The LAMP assay confirmed that the *ail*-positive samples contain DNA of pathogenic *Y. enterocolitica* strains. The LAMP result from the direct dye

visualization with hydroxynaphthol blue was identical to the result from the DNA electrophoresis in agarose gel but this step was skipped and the whole procedure was shorten by two hours.

In conclusion, our findings indicate that the examined groups of pigs carry pathogenic *Y. enterocolitica* on their tonsils at the time of slaughter which has an importance for the health risk of consumers. The LAMP method is reliable, fast, and selective. Further optimisation of the DNA isolation protocol is needed in order to enhance its sensitivity after direct DNA isolation. Hence, it could be applied in combination with the ISO standard because it is cheaper and faster than the conventional PCR method as far as there is no need for a thermocycler machine and gel electrophoresis.

Belgian-Bulgarian Bilateral project

References:

Zaharieva M. M., Gatzovska M., van Collie E., Heyndricks M., Najdenski H. Detection of *Yersinia enterocolitica* in pig faeces by using loop mediated DNA amplification Workshop on food-borne pathogens and food safety, 26.05.2016 - 27.05.2016, Sofia, Bulgaria - Pre-Conference Meeting for International Conference on Food Microbiology, Birmingham, UK, August 08-10, 2016.

Zaharieva M.M., Gatzovska M., Teneva V., van Collie E., Heyndricks M., Najdenski H. Detection of *Yersinia enterocolitica* in pig faeces and tonsils by LAMP 3rd Institut Pasteur International Network Symposium "From Basic Sciences to Biomarkers & Tools in Global Health", 29.11.2016 - 02.12.2016, Paris, France.

STUDIES ON *LACTOCOCCUS GARVIEAE*

Project supervisor – P. Orozova, PhD, National Diagnostic and Research Veterinary Institute "Prof. Georgi Pavlov", Sofia, Bulgaria

***Project leader*:** H. Najdenski, DSc

Project collaborators: Medical University, Sofia; Faculty of Biology, Sofia University "St. Kliment Ohridski"

***Project staff*:** I. Tsvetkova, MS

Lactococcus garvieae is the aetiologic agent of Lactococcosis, an emerging disease that affects many species of fish and causes major economic losses in aquaculture. It affects both saltwater and freshwater fish when the water temperature rises above 16ºC in the summer months being a major problem in rainbow trout farming. It has been isolated in yellow tail (*Seriola quinqueradiata*) in Japan and from rainbow trout (*Onchorhynchus mykiss*) in European countries like Spain, UK, Portugal, Italy, France, etc. and Turkey. In the frame of the recent project more than 200 fish samples were collected and all isolates suspected for *L. garvieae* were characterized by their morphology, Gram staining, presence/absence of capsule, agglutination with polyvalent antigens and antimicrobial resistance. The isolates were confirmed by PCR method previously optimized in the Referent Laboratory of the National

Diagnostic and Research Veterinary Institute "Prof. Georgi Pavlov". Identification was carried out by BIOLOG GEN III system (Biolog, Hayward, CA, USA). Direct PCR was developed by using DNA isolated from the brain of fish (EUREX kit, E3551) and primers ITSLg 30F and ITSLg319R. Organs of trout with a lactococcal clinic features have been prepared for histological examination.

SYNTHESIS, ANTIMYCOBACTERIAL ACTIVITY OF NOVEL HYDRAZIDE-HYDRAZONE DERIVATIVES WITH 2H-CHROMENE AND COUMARINE SCAFFOLD

Project leather: V. Angelova, PhD, Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia

Research staff: V. Valcheva, PhD

A new convenient method for preparation of 2-aroyl-[1]benzopyrano[4,3-c]pyrazol-4(1H)-one derivatives 5b–g and coumarin containing hydrazide-hydrazone analogues 4a–e was presented. The antimycobacterial activity against reference strain Mycobacterium tuberculosis H37Rv and cytotoxicity against the human embryonic kidney cell line HEK-293 were tested in vitro. All compounds demonstrated significant minimum inhibitory concentrations (MIC) ranging 0.28–1.69 lM, which were comparable to those of isoniazid. The cytotoxicity (IC50 > 200 mM) to the ‘‘normal cell" model HEK-293T exhibited by 2-aroyl- [1]benzopyrano[4,3-c]pyrazol-4(1H)-one derivatives 5b–e, was noticeably milder compared to that of their hydrazone analogues 4a–e (IC50 33–403 mM). Molecular docking studies on compounds 4a–e and 5b–g were also carried out to investigate their binding to the 2-trans-enoyl-ACP reductase (InhA) enzyme involved in M. tuberculosis cell wall biogenesis. The binding model suggested one or more hydrogen bonding and/or arene-H or arene-arene interactions between hydrazones or pyrazole-fused coumarin derivatives and InhA enzyme for all synthesized compounds.

Joint research project between Medical University of Sofia and The Stephan Angeloff Institute of Microbiology, BAS

References:

Angelova V., Valcheva V., Pencheva T., Voynikov Y., Vassilev N., Mihaylova R., Momekov, G., Shivachev B. Synthesis, antimycobacterial activity and docking study of 2-aroyl-[1] benzopyrano [4, 3-c] pyrazol-4 (1H)-one derivatives and related hydrazide-hydrazones. *Bioorg. Med. Chem. Lett*. 2016 (in press).

Angelova V., Valcheva V., Vassilev N.G., Buyukliev R., Momekov G., Dimitrov I,. Saso L., Djukic M., Shivachev B. Antimycobacterial activity of novel hydrazide-hydrazone derivatives with 2H-chromene and coumarin scaffold. *Bioorg. Med. Chem. Lett.* 2016 (in press).

INVESTIGATION OF EXTRACTS AND BIOACTIVE SUBSTANCES OF *GEUM URBANUM* L. AS DRUG CANDIDATES WITH ANTIMICROBIAL AND ANTI-NEOPLASTIC ACTIVITY

Project supervisor: M. M. Zaharieva, PhD

Project leader: L. Dimitrova, MS

Scientific consultant: H. Najdenski, DSc

Antimicrobial resistance in pathogenic bacteria leads to increased morbidity and mortality due to the occurrence of chronic infections, superinfections and increased risk of carcinogenesis. The aim of this study was to evaluated the antimicrobial and antineoplastic properties of new natural drug candidates with a favorable toxicological profile isolated from *Geum urbanum* L. According to the ethnopharmacological data, this medicinal plant was used from ancient time for treatment of gastrointestinal disorders and diseases, associated with feverishness and inflammations.

The subjects of our study were MeOH extracts and their fractions (petroleum ether, EtOAc and *n*-BuOH), and crude H_2O and 20% EtOH[1] extracts of roots and aerial parts of *G. urbanum,* extracted in 2016. Minimal inhibitory, minimal bactericidal concentrations and determination of the respiratory activity was estimated on a panel of Gram-(+) and Gram-(-) pathogenic bacteria and *Candida albicans* by broth microdilution method, according to the requirements of EUCAST, ISO 20776/1-2006 and CLSI. The inhibition effects of some fractions on the phenotypes associated with the quorum sensing system of *Chromobacterium violaceum* 026 and *Pseudomonas aeruginosa* 01 (swarming motility and synthesis of pyocyanin and violacein) were established. DPPH and SOD methods were used for radical scavenging evaluation of extracts and their fractions. The total phenolic content was determined quantitative and qualitative. The cytotoxic potential of all extracts and compounds was determined on the cell lines HEK-293 (transformed human embryonic kidney cells), T-24 and BC-3C (carcinoma of the bladder) and HEP-G2 (hepatocellular carcinoma). The EtOAc fraction of roots showed the highest radical scavenging activities and the highest content of polyphenolic compounds (61 %). Seven individual compounds were isolated previously from this extract and their structures were elucidated by comparison of their spectral characteristics (^{1}H and ^{13}C NMR, MS) with literature data: tormentic acid (1), 3-O-methylellagic acid-3′-O-α3″-O-acetylrhamnopyranoside (2), 3-O-methylellagic acid-3′-O-α-2″-O-acetylrhamnopyranoside (3), catechin (4), 3,3′-di-O-methylellagic acid-4-O-β-dglucopyranoside (5), niga-ichigoside F1 (6) and gein (7). The isolated copmounds (2) and (3) were found for the first time in the genus *Geum* and three others (1), (5) and (6) were newly detected for the species *G. urbanum*. The EtOAc extract of *G. urbanum* aerial parts showed a high antibacterial activity against *Staphylococcus aureus, S. epidermidis* and *Bacillus cereus* (39 – 313 μg/ml) and lower cytotoxicity on HEK-293 (IC_{50} (72 h) = 65.05 μg/ml) and HEP-G2 (IC_{50} (72 h) = 77.11 μg/ml) compared to the other cell lines (IC_{50} (72 h) ranging from 17.29 to 46.8 μg/ml). Compound (1) showed bacteriostatic and fungistatic activity against *S. aureus* and *C. albicans* (125 μg/ml and 500 μg/ml, respectively), but was highly cytotoxic against HEK-293 (IC_{50} (72 h) 21.03 μg/ml).

In conclusion, *G. urbanum* L. is a perspective medicinal plant and deserves further more detailed studies.

The obtained results were presented at 6th Congress of Pharmacy with

International Participation, 13-16.10.2016, Sandanski, Bulgaria.

Grant DFNP-70, supported by "Programme for Carrier Development of Young Scientists", BAS

SYNTHESIS, ANTIMYCOBACTERIAL ACTIVITY OF ISOBORNYLAMINE AND BORNYLAMINE DERIVED AMIDES

Project leather: G. Stavrakov, Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia

Research staff: V. Valcheva, PhD

A series of ten novel amides was designed and synthesized on the base of the camphane scaffold by coupling of isobornylamine and bornylamine with different carboxylic acids. The compounds were screened for their in vitro activity against M. tuberculosis H37Rv and cytotoxic activity against the human embryonal kidney cell line HEK-293T. Six of the structures revealed profound anti-tuberculosis activity (MICs up to 0.16 μM) in combination with moderate to low cytotoxicity. The compound derived from bornylamine and furan-2-carboxylic acid can be considered as a promising lead for the development of anti-mycobacterial agents.

Joint research project between Medical University of Sofia and The Stephan Angeloff Institute of Microbiology - BAS

References:

Stavrakov G., Valcheva V., Voynikov Y., Philipova I., Atanasova M., Konstantinov S., Doytchinova I. Design, synthesis, and antimycobacterial activity of novel theophylline-7-acetic acid derivatives with amino acid moieties. *Chem Biology & Drug Design.* 2016, 87(3), 335-341.

Stavrakov, G., Philipova, I., Valcheva, V., Momekov, G. Isobornylamine and bornylamine derived amides–synthesis, antimycobacterial activity and cytotoxicity. *Bulg Chem. Commun*. 2016, 48(1), 43-49.

STUDY ON THE PRESENCE OF PARATUBERCULOSIS INFECTION IN CATTLE REACTED POSITIVELY OR DOUBTFULLY TO PPD TUBERCULIN

***Project supervisor*:** T. Savova, PhD, National Diagnostic and Research Veterinary Institute „Prof. G. Pavlov", Sofia

***Project leader*:** M. Bonovska, PhD

***Project collaborators*:** National Diagnostic and Research Veterinary Institute „Prof. G. Pavlov", Directorate "Animal Health and Animal Welfare and Feed Control", Bulgarian Food Safety Agency

***Research staff*:** St. Atanasova, PhD; A. Dimitrova, PhD; V. Valcheva, PhD; Y. Petkov, PhD; N. Lalkovski, PhD; R. Kolev,MS; R. Tsvetkova, MS

Etiological agent of paratuberculosis in animals (John's disease) is *Mycobacterium avium* subsp. *paratuberculosis* (Map). The disease mostly affects young domestic and wild ruminants and may be chronic or acute. In domestic ruminants the acute form usually caused enteritis, diarrhea, weakness and progressive emaciation that can lead to death the affected animals and large economic losses. Diagnosis of John's disease is a difficult and very long process because there are no standart diagnostic methods for detecting both forms of the disease - clinical and subclinical. Because its rare clinical manifestations, the incidence of subclinical forms of the disease is not investigated or monitored. In official veterinary statistics in Bulgaria accurate data on the spread of the disease in animals are missing. There is no National Program for targeted research on paratuberculosis in ruminants. Only single cases in cattle in private farms and in wild deer and mouflons are described. The absence of information about John's disease in our country requires more systematic and in-depth research in this area. The used diagnostic tuberculin skin test often gives false positive reactions, based on the antigenic similarity between mycobacteria. To avoid cross-reaction, it is necessary to deepen the studies of paratuberculosis in herds reacted to the avian PPD tuberculin. For the purpose lymph nodes of 45 cattle from five farms in different regions of the country, reacted positively or doubtfully to bovine and avian PPD tuberculin, were examined. The tested tissue samples showed no specific growth of *M. paratuberculosis* (MAP) after three-month cultivation in Middlebrook 7H9 broth and on solid Herrold's medium with mycobactin and pyruvate. All samples were negative in a conventional IS*900* PCR. By incoming lab diagnostic materials (internal organs and lymph nodes) of 12 cattle, 3 were observed patho-morphological changes characteristic of paratuberculosis. MAP was confirmed bacteriologically and by the IS*900* PCR with a conventional kit. PCR analysis showed the presence of specific 209-bp fragment.

Single cases of paratuberculosis in cattle found in the survey period proved that the disease exists in Bulgaria. The results show the need for further research on the problem farms to determine the prevalence of paratuberculosis in the bovine population in the country.

Joint research project between National Diagnostic Research Veterinary Institute and The Stephan Angeloff Institute of Microbiology, BAS

References:

Savova T., Bonovska M., Dimitrova A., Lalkovski N. Differentiation of mycobacteria from *Mycobacterium avium* complex. *Animal Sci.* 2016, LIII, 155-162.

LABORATORY OF GENETICS AND RESISTANCE OF MYCOBACTERIA

Final Summary

INNOVATIVE MODEL FOR TESTING ANTIBACTERIAL EFFECTS ON MYCOBACTERIA AND L-FORMS: PERSPECTIVES FOR EFFECTIVE TREATMENT OF RECCURENT MYCOBACTERIOSES

Project leader: G. Slavchev, PhD

Project supervisor: N. Markova, MD, PhD

Mycobacterioses are widespread worldwide. Their treatment is difficult and requires long-term therapeutic courses. In this connection, the goal of the current project was to be created an original model presented by *Mycobacterium avium subsp paratuberculosis* (MAP) and its L-forms in order to test *in vitro* effects of antimycobacterial agents with rapid genetic assay. .An experimental model for reproducible induction and production of mycobacterial L-forms was developed using cryogenic effects *in vitro*. The morphological features of L-forms were characterized by light and scanning electron microscopy. An original protocol for quantitative evaluation of antimycobacterial effect on mycobacteria and L-forms with RT-qPCR was developed. Some of the experimental parameters were applied in a model of *Mycobacterium tuberculosis* and L-forms with observed drug tolerance to ethambutol.

Grant DFNP-63/27.04., supported by "Programme for Carrier Development of Young Scientists", BAS

References:

Slavchev, G., Michailova, L., Markova, N. (2016). L-form transformation phenomenon in Mycobacterium tuberculosis associated with drug tolerance to ethambutol. *Int. J. Mycobacteriol*. 5, 454-459.

New project

PROFILE OF γ-δ T LYMPHOCYTES DURING NORMAL PREGNANCY AND PLACENTOBIOME IN BCG VACCINATED PREGNANT WOMEN

Project leader: T. Dimova, PhD, Institute of Biology and Immunology of Reproduction

Research staff: N. Markova, MD, PhD; A. Cherneva, MS; A. Terzieva; L. Djerov, A. Nikolov

Project collaborators: Institute of Biology and Immunology of Reproduction; Medical University, Sofia

Grant ID H 03/9 -2016 of the Bugarian Science Fund, Ministry of Education and Science

Reference:

Markova, N., Slavchev G., Djerov L., Nikolov A., Dimova T. Mycobacterial L-forms are found in cord blood: A potential vertical transmission of BCG from vaccinated mothers. *Hum. Vaccin. Immunother*. 2016, 12, 2565-2571.

Final Summary

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 collaborator: Gebze Institute of Technology, Turkey; Institute of Organic Chemistry with Centre of Phytochemistry,

Research staff: V. Kussovski, PhD

Axially di-(alpha,alpha-diphenyl-4-pyridylmethoxy) silicon(IV) phthalocyanine (3) and its quaternized derivative (3Q) were synthesized and tested as photosensitizers against tumor and bacterial cells. These new phthalocyanines were characterized by elemental analysis, and different spectroscopic methods such as FT-IR, UV–Vis, MALDI-TOF and 1H NMR. The photophysical properties such as absorption and fluorescence, and the photochemical properties such as singlet oxygen generation of both phthalocyanines were investigated in solutions. The obtained values were compared to the values obtained with unsubstituted silicon(IV) phthalocyanine dichloride (SiPcCl2). The addition of two di-(alpha,alpha-diphenyl-4-pyridylmethanol) groups as axial ligands showed an improvement of the photophysical and photochemical properties and an increasement of the singlet oxygen quantum yield (UD) from 0.15 to 0.33 was determined. The photodynamic efficacy of synthesized photosensitizers (3 and 3Q) were evaluated with promising photocytotoxicity (17% cell survival for 3 and 28% for 3Q) against the cervical cancer cell line (HeLa). The photodynamic inactivation of pathogenic bacterial strains Streptococcus mutans, Staphylococcus aureus, and Pseudomonas aeruginosa suggested a high susceptibility with quaternized derivative (3Q). The both Gram-positive bacterial strains were fully photoinactivated with 11 lM 3Q and mild light dose 50 J.cm_2. In case of P. aeruginosa the effect was negligible for concentrations up to 22 lM 3Q and light dose 100 J.cm_2. The results suggested that the novel axially substituted silicon (IV) phthalocyanines have promising characteristic as photosensitizer towards tumor cells. The quaternized derivative 3Q has high potential for photoinactivation of pathogenic bacterial species.

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

References:

Ömeroğlu I., Kaya E. N., Göksel M., Kussovski V., Mantareva V., Durmuş M. Axially substituted silicon (IV) phthalocyanine and its quaternized derivative as photosensitizers towards tumor cells and bacterial pathogens. *Bioorg. Med. Chem.* 2016 (in press).

Kussovski V., Mantareva V., Durmuş M., Angelov I. Quaternized Zn(II) phthalocyanines for photodynamic strategy against resistant periodontal bacteria. *Z. Naturforsch*. 2016 (in press).

DEPARTMENT OF APPLIED MICROBIOLOGY

LABORATORY OF MICROBIAL BIOSYNTHESIS AND ECOLOGY

Current project

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

Project collaborators: West Parana State University, Department of Chemical Engineering

Research staff: A. Kroumov, PhD

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 2016 was published 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 algae strains were isolated from Brazilian lakes and preliminary morphological identification was performed. Their identification is in progress by using modern molecular DNA methods. Preparation of articles on the subject is in progress.

Grant "Special Visiting Researcher" under the program "Science without Borders", Process # 313737/2014-4, CNPq/MCTI.

References:

Kroumov, A. D., Módenes, A. N., Trigueros, D. E. G., Espinoza-Quinones, F. R., Borba, C. E., Scheufele, F. B., Hinterholz, C. L. (2016). A systems approach for CO_2 fixation from flue gas by microalgae–theory review. *Process Biochem*. 51, 11, 1817-1832.

Schuelter A. Isolation and identification of microalgae species by using modern molecular methods. West Parana State University, Toledo PR, Brazil.

LABORATORY OF EXTREMOPHILIC BACTERIA

Final summary

CHARACTERIZATION OF ARCHAEAL DIVERSITY IN BULGARIAN HOT SPRINGS

Project supervisor: 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. Vassilev, MSc.

Metagenomic analysis of the biotechnological potential of microorganisms in Bulgarian hot springs was performed for the first time. Gene sequences for cellulases, xylanases, α-amylases, chitinases, lipases and α-mannosidases were identified in metagenomes from seven Bulgarian hot springs. As some of these genes showed high degree of novelty they represent a potential for novel products that could be industrially explored. A culture independent approach based on the analysis of nearly complete archaeal 16S rRNA genes revealed an unusually high archaeal diversity in some of the hottest Bulgarian geothermal springs, Vlasa, Levunovo and Vetren dol. Young Bulgarian scientist, PhD student Nikoleta Boteva transferred the experience of Norwegian scientists on cultivation of thermophilic anaerobic Archaea in Bulgarian lab. The work on isolation of pure cultures and their characterization currently continue by the new equipment provided by the project funding.

Grant D03-100/05.06.2015 funded by the Financial Mechanism of the European Economic Area, Projects for Inter-Institutional Cooperation Measure.

Current projects

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

Project supervisor: M. Kambourova, DSc

Project collaborator: University of Food Technologies, Plovdiv

Research staff: N. Radchenkova, PhD; I. Boyadzhieva, PhD; N. Boteva, PhD student; N. Atanasova, MSc; E. Tonkova-Vasileva, PhD; S. Vassilev, MSc

Both archaeal and bacterial diversity was characterized in Pomorie Saltlerns and unusually high number of presented taxa in hypersaline environment was established. Additionally, domination of species not previously reported as dominant and large proportion of unknown sequences were observed. Isolated 29 bacterial strains from the Pomorie and Burgas salt mines and the Atanasovsko Lake referred to 11 species

from eight genera allocated into the phyla *Proteobacteria*, *Firmicutes* and *Actinobacteria.* Among the isolates producers of nine different enzymes were identified. Four of the strains were able to synthesize exopolysaccharides best producer being *Chromohalobacter canadensis* 28. A number of functionally valuable properties have been identified for this EPS (water solubility, surface activity of aqueous solutions, thermostability, viscosity, optical activity, emulsifying and stabilizing ability of oil-aqueous emulsions) that make it attractive for future applications.

Grant 02-26/2014 of the Bugarian Science Fund, Ministry of Education and Science

References:

Kambourova, M., Tomova, I., Boyadzhieva, I., Radchenkova, N., Vasileva-Tonkova, E. Phylogenetic analysis of the bacterial community in a crystallizer pond, pomorie salterns, Bulgaria. *Biotechnol. Biotechnol. Equip.* 2016, 31, 325-332.

Kambourova, M., Tomova, I., Boyadzhieva, I., Radchenkova, N., Vasileva-Tonkova, E. Unusually high archaeal diversity in a crystallizer pond, Pomorie Salterns, Bulgaria, revealed by phylogenetic analysis. *Archaea* Article 2016ID 7459679, 9 pages.

EXOPOLYSACCAHARIDE 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

Among the halophilic microorganisms, isolated from Bulgarian salt niches ten halophilic bacterial strains were selected as probable exopolysaccharide (EPS) producers. The high levels of synthesis and interesting functional properties of EPS from *Chromohalobacter canadensis* strain 28 determined our interest in further studies with this strain. The production environment of EPS was optimized in terms of quantitative composition. Maximum synthesis was observed in a medium with sucrose, peptone and 15% salt. Among the physico-chemical conditions, the highest synthesis and the highest specific growth rate μ were found at pH 7.5. For further characterization, EPS was purified by ethanol precipitation of the supernatant, dialysis to remove the salt and residual sugars, and ion-exchange chromatography coupled with gel chromatography of DEAE Sepharose 6B. The purified EPS was sent for NMR analysis at the Institute of Chemistry of Biomolecules, CNR, Naples, Italy. PhD student Alessia Gioiello, Institute of Chemistry of Biomolecules, CNR, Italy, worked in Sofia with the team of Extremophilic Bacteria, Institute of Microbiology, BAS in the period 02.11-02.12.2016. Assoc. Prof. Margarita Kamburova and Assist. Prof. Nadya Radchenkova visited the Institute of Chemistry of Biomolecules, CNR, Italy, in the period 25-31.10.2016.

Joint research project between CNR, Italy and BAS, Bulgaria

METABOLIC PROFILING AND ANTINEOPLASTIC POTENTIAL OF ANTARCTIC YEASTs

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

Project leader: S. Rusinova-Videva, PhD

The report on the project could be find in the reports of the Laboratory Applied Biotechnologies to which the young scientist belongs.

Grant 60/27.04.2016, supported by "Programme for Carrier Development of Young Scientists", BAS

LABORATORY OF APPLIED BIOTECHNOLOGIES

Final summary

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

Project leader: M. I. 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: A. 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:

Marchev, A., Dinkova-Kostova, A. T., Gyorgy, Z., Mirmazloum, I., Aneva, I. Y., Georgiev, M. I. *Rhodiola rosea* L.: from golden root to green cell factories. *Phytochem. Rev.* 2016, 15, 515-536.

Skalicka-Wozniak, K., Orhan, E., Georgiev, M. I. Adulteration of herbal sexual enhancers and slimmers: the wish for better sexual well-being and perfect body can be risky. *Food Chem. Toxicol.* 2016, (in press).

Chen, L., Teng, H., Xie, Z., Cao, H., Cheang, W. S., Skalicka-Wozniak, K., Georgiev, M. I., Xiao, J. Modifications of dietary flavonoids towards improved bioactivity: An update on structure-activity relationship. *Crit. Rev. Food Sci. Nutr.* 2016, 1-15

BIOTECHNOLOGICAL PLATFORM FOR SUSTAINABLE PRODUCTION OF PHARMACEUTICALLY RELEVANT METABOLITES FROM GOLDEN ROOT

Project leader: A. Marchev, PhD

Project staff: M. I. Georgiev, PhD

Rhodiola rosea L. (roseroot or golden root) family Crassulaceae, is a multipurpose medicinal plant with well-established adaptogenic properties, able to increase the body's nonspecific resistance and normalize its functions in response to different stressors of emotional, mental, and physical origin. *R. rosea* extracts, as well as, its valuable pharmacologically active substances, such as salidroside, tyrosol, and rosavins, are extensively studied for their neuroprotective, hepatoprotective, antioxidant, antiviral, anticancer and anti-inflammatory activities. The main goal of the project is to perform metabolite profiling and study the metabolic differences in rhizomes, roots and aerial parts of the medicinal plant *R. rosea* wild-grown in Bulgaria and its *in vitro* cultures, as well as, evaluation of their antineoplastic effect towards different hematologic neoplasms.

Grant DFNP-58, supported by "Programme for Carrier Development of Young Scientists", BAS

References:

Marchev, A., Dimitrova P., Koycheva, I., Georgiev, M. Altered expression of TRAIL on mouse T cells via ERK phosphorylation by *Rhodiola rosea* L. and its marker compounds. *Food Chem. Toxicol.* 2016, (in press).

Marchev A., Aveva I., Koycheva I., Georgiev M. Phytochemical variations of *Rhodiola rosea* L. wild-grown in Bulgaria. *Phytochem. Lett.* 2016, 20, 386-390.

Current projects

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

Project leader: M. I. Georgiev, Ph.D

Project collaborators: Institute of Organic Chemistry with Centre of Phytochemistry-BAS; University of Sofia "St. Kliment Ohridski"

Research staff: P. Dimitrova, PhD; A. Marchev, PhD; S. Rusinova-Videva, PhD; V. Milanova, G. Zahmanov, E. 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 of the Bugarian Science Fund, Ministry of Education and Science

References:

Marchev, A., Yordanova, Z., Alipieva, K., Zahmanov, G., Rusinova-Videva, S., Kapchina-Toteva, V., Simova, S., Popova, M., Georgiev, M. I. Genetic transformation of rare *Verbascum eriophorum* Godr. plants and metabolic alterations revealed by NMR-based metabolomics. *Biotechnol. Lett.* 2016, 38, 1621-1629.

Marchev, A., Georgiev M. I. Plant cell bioprocesses. In: Current Developments of Biotechnology and Bioengineering. Bioprocess, Bioreactors and Controls. (Larocche C., Sanroman M., Du G., Pandey A., Eds.), Elsevier, 2016, pp. 73-95.

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:

Georgiev, M. I., Radziszewska, A., Neumann, M., Marchev, A., Alipieva, K., Ludwig-Müller, J. Metabolic alterations of *Verbascum nigrum* L. plants and SAArT transformed roots as revealed by NMR-based metabolomics. *Plant Cell Tiss. Organ Cult.* 2015, 123, 349-356.

PREPARATION OF FERMRNTED DAIRY PRODUCTS WITH ENHANCED BIOLOGICAL VALUE

Project supervisor: Dora Beshkova, PhD

Project leader: T. Teneva-Angelova, PhD student

Lactic acid bacteria isolated from alternative sources (medicinal plants), possessing different biological activities and functional properties are promising and new effective sources, satisfying the needs of the consumer for functional food. They can be used in a variety of dietary foods in order to maintain human health and to act like prevention against various diseases.

The project aims is formation and characterization of new bio-consortium starters, containing lactic acid bacteria, isolated from the medicinal plants Salvia blepharophylla L. and Panax ginseng C.A.Meyer for production of foods with increased health benefits.

The subject of our research are strains Streptococcus thermophilus and Lactobacillus rhamnosus isolated from Salvia blepharophilla Brandegee ex Epling and Panax ginseng C.A.Meyer. From 7 plant-derived Streptococcus thermophilus strains and 25 Lactobacillus rhamnosus strains were selected the strains Streptococcus thermophilus SbfGER352 and Lactobacillus rhamnosus PglGER39, which have improved physiological and biochemical characteristics (coagulation time, lactic acid production, growth in a wider pH and temperature range, halotolerance, resistance of LAB against extracts from medicinal plants).

These strains were also characterized (acidification, viable cells concentration, lactic acid production, presence of biological activities, coagulation process rate, using optical system) in order to include them as components of newly formed starters for fermented dairy products with increased health benefits.

Grant DFNP-62, supported by "Programme for Carrier Development of Young Scientists", BAS

<u>Pending support</u>

COMPLEX ASSESSMENT OF BULGARIAN TOMATOES AND DEVELOPMENT OF NEW CULTIVARS WITH IMPROVED ANTIOXIDANT ACTIVITY OF THEIR FRUITS

***Project supervisor*:** B. Bojinov, Agricultural University, Plovdiv

***Project leader*:** A. Pavlov, DSc

APPLICATION OF OMICS TECHNOLOGIES FOR INVESTIGATION HEALTH POTENTIAL OF BULGARIAN HONEY

Project supervisor: M. Shishinova, PhD, Sofia University

Project leader: A. Pavlov, DSc

New project

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

Project leader: M. I. 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: A. Marchev, PhD; I. Koycheva, Msc

PlantaSYST aims at establishing a new Center of Plant Systems Biology and Biotechnology (CPSBB) in Plovdiv, Bulgaria. The CPSBB is registered as an autonomous legal research entity during TEAMING Phase I and is firmly supported by the Bulgarian and German PlantaSYST partners, the Governments of Bulgaria and Germany, and the Plovdiv Municipality. The aim of the project is to position the Center as a leading research organization in Bulgaria and South-East Europe by implementing cutting-edge genetics, functional genomics, metabolomics and bioinformatics technologies in order to unravel the plant biochemical machinery and translate the scientific knowledge into the food market and industrial applications for development of value-added products with potential markets in medicine, pharmacy and cosmetics.

Grant PlantaSYST – SGA/CSA: 739582 – under FPA: 664620, H2020, WIDESPREAD-2016-2017/H2020-WIDESPREAD-01-2016-2017-TeamingPhase2

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. Naidenski, DSc; M. Angelova, DSc; E. Chorukova, PhD; E. Krumova, PhD; V. Kasovski, PhD,; L. Kabaivanova, PhD; N. Kostadinova, PhD; V. Hubenov, PhD; S. Mihaylova, MS; L. Dimitrova, PhD student

After examining the chemical composition of used lignocellulosic waste, an optimal nutrient medium have been developed and C/N ratio for laboratory cultivation of the microbial community was chosen.

Anaerobic biodegradation for the production of methane in a pilot bioreactor from three lignocellulosic wastes specific to our country (with and without pre-treatment) have been done. The stable processes were achieved in all three wastes after adaptation of the microbial community.

Software sensors have been developed to assess the non-measurable variables of a two-phase process of anaerobic biodegradation of organic waste for the simultaneous production of hydrogen and methane.

Grant DFNI-E02/13 of the Bugarian Science Fund, Ministry of Education and Science

References:

Simeonov, I., Denchev, D., Kabaivanova, L., Kroumova, E., Chorukova, E., Hubenov, V., Mihailova, S. Different Types of Pretreatment of Lignocellulosic Wastes for Methane Production. *Bulg. Chem. Comm*. 2016 (in press).

Kabaivanova, L., Simeonov, I., Denchev D., Mihaylova S., Hubenov V., Nikolova R., Todorova D. Thermochemical pretreatment of lignocellulosic substrates for anaerobic digestion at mesophilic and thermophilic conditions. *Ecol. Eng. Environ. Prot*. 2016, 2, 42-49.

Chorukova, E., Diop, S., Simeonov, I., Tebbani, S. Modeling and software sensors design for anaerobic digestion process. *Ecol. Eng. Environ. Prot*. 2016, 2, 62-72.

Denchev, D., Hubenov, V., Simeonov, I., Kabaivanova, L. Biohydrogen production from lignocellulosic waste with anaerobic bacteria. Proc. of the 4th International Conference on Water, Energy and Environment (ICWEE), June 1-2, 2016, Burgas, Bulgaria, 7-12.

Simeonov, I., Chorukova, E. Mathematical modeling of the anaerobic digestion with production of hydrogen and methane. Proc. 4th International Conference on Water, Energy and Environment (ICWEE), June 1-2, 2016, Burgas, Bulgaria, 32-38.

Borisov, M., Dimitrova, N., Simeonov, I. Mathematical modelling of anaerobic digestion with hydrogen and methane production. Preprints of the 6th IFAC Conference on Foundations of Systems Biology in Engineering, The International Federation of Automatic Control, October 9-12, 2016, Magdeburg, Germany.

Kabaivanova, L., Simeonov, I., Denchev, D., Hubenov, V., Mihaylova, S. Anaerobic co-digestion of cattle manure and pretreated wheat straw at two temperature regimes for

biomethane production, "Ecology and health" Conference, June 9-10, 2016, Plovdiv, Bulgaria, 424-428.

Simeonov, I., Denchev, D., Kabaivanova, L., Hubenov, V., Mihaylova, S., Chorukova, E., Krumova, E. Utilization of lignocellulosic wastes by anaerobic digestion for methane production. 4th Biotechnology World Congress, February 15–18, 2016, Dubai, UAE.

Lakov, V., Chorukova, E, Simeonov, I. Extremum seeking control of a cascade of two anaerobic bioreactors for production of hydrogen and methane. Automatics and informatics, October 4-5, 2016, Sofia, Bulgaria, pp. 81-85.

Akivanov, V., Lakov, V., Simeonov, I. Automation of the processes of anaerobic digestion of organic wastes with production of hydrogen and methane. *J. Automat. Informat*. 2016, 1, 3-8.

ERASMUS+

Project supervisor: N. Christov, PhD

Project leader: I. Simeonov, PhD

Research staff: V. Hubenov, PhD

Mobility program for training in University of Lille 1 – Science and Technology, UFR IEEA (Cité Scientifique, 59655 Villeneuve d'Ascq Cédex, France), 2-6 May 2016. Overall objectives of the mobility: Optimisation of biotechnological systems for production of biohydrogen and biomethane from lignocellulosic wastes. Influence of ultrasound on the biogas production.

Grant: European Union

DEPARTMENT OF VIROLOGY

Final summary

A NEW APPROACH FOR HIGHLY EFFICIENT CHEMOTHRAPY OF ENTEROVIRAL INFECTIONS

Project leader: A. S. Galabov, DSc

Project staff: I. Nikolova, PhD; A. Stoyanova, MS; P. Grozdanov, PhD; Y. Abashev, PhD; S. Philipov, PhD; L. Mukova, MS; N. Nikolova, MS; P. Stoyanova, DVM; I. Zahova; E. Dimitrova

As a continuation of previous investigations it was studies the effect of a combination of three enterovirus replication inhibitors - pleconaril, MDL-860 and oxoglaucine applied following the original proposed by us treatment scheme „consecutive alternating administration" (CAA) on experimental neuroinfection by Coxsackievirus B1 in mice. It was established: (i) a complete hamper the development of drug resistence; (ii) a phenomenon of increased susceptibility to the effect of antivirals included in the combination; (iii) a pronounced protective effect (from 31.3% to 68% depending of the pleconaril dose; (iv) expressed suppression of the virus titre (by 4-5 logs). These results represent new proofs in support of CAA treatment scheme as a perspective of the development of clinically effective chemotherapy of enteroviral infections. Related with this problematics is the develoipment od a PhD dissertation as well as a project of helping of young scientists sponsored by BAS. A common work with Enterovirus Laboratory of Institut Pasteur Paris is in course. A cooperation with US Cilead company (San Francisco) was started.

Grant Б-01-13/12 of the Bugarian Science Fund, Ministry of Education and Science

BALKAN ENDEMIC NEPHROPATHY

Project leaders: A. S. Galabov, DSc

Project collaborators: Department of Medical Genetics, Medical University of Sofia; Serbian Academy of Sciences and Arts (Belgrade-Nish); Macedonian Academy of Sciences and Arts (Skopje)

Research staff: D. Toncheva, DSc, R. Staneva, PhD; A. Pashov, PhD; Tz. Dimitrov, DSc; M. Polenakovic, PhD; R. Cukuranovic, PhD, L. Jankovic-Velickovic, PhD, I. Ignjatovic, PhD; Z. Radovanovic, PhD, L. Djukanovic, PhD, M. Kovacevic, PhD

Preparing and editing of the monograph "Balkan Endemic Nephropathy – a Comprehensive View", ISBN 978-619-7177-03-9 (Press Product Line Ltd., Sofia, 2016). Editors-in-Chief: A. S. Galabov,

DSc, D. Toncheva, DSc, M. Polenakovic, PhD.

Interacademic projects BAS-SASA, BAS-MASA.

Chapters in the monograph:

Galabov, A. S. Role of viruses in the etiology and pathogenesis. – In: Balkan Endemic Nephropathy/A Comprehensive View (V. Stefanovic et al., Eds). Sofia, Press Product Line, 2016, pp. 105-130.

Galabov, A. S., Pashov, A. Immunologic mechanisms. -In: Balkan Endemic Nephropathy / A Comprehensive View (V. Stefanovic et al., Eds).Sofia, Press Product Line, 2016, pp. 131-140.

STUDY OF THE ROLE AND POTENTIAL OF ONCOLYTIC VIRUSES IN THE PROCESSES OF TUMOR DESTRUCTION

Project supervisor: J. Rommelaere, Deutsches Krebsforschung Zentrum, Heidelberg

Project collaborators: Deutsches Krebsforschung Zentrum, Heidelberg; The Stephan Angeloff Institute of Microbiology, BAS

Research staff: A. Angelova, PhD; Z. Raykov, PhD; A. S. Galabov, DSc

Eighteen patients with neuroglyoma in the Clinics of Neurosurgery, University of Heidelberg, were treated with parvovirus H1 adminsitred intravenously and applied intra tumors, the tumor being removed 10 days after virus inoculation. It was established a statistically significant lengthening of the tumor remission. This effect was substantially strengthened in combination with the immunomodulator Avastin.

Common project of Deutsches Krebsforschung Zentrum, Heidelberg and The Stephan Angeloff Institute of Microbiology, BAS (2005 – 2016).

References:

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Vassileva-Pencheva, R., A. S. Galabov (2016). Effectiveness of the consecutive alternative administration course of a triple antiviral combination in Coxsakievirus B3 infections in mice. *Drug Research* 66 (12), 639-643.

Galabov, A. S., Stoyanova, A. (2016). Consecutive alternating administration of antiviral combinations: a novel treatment approach against Coxsackievirus B1 neuroinfection. J. Antivir. Antiretrov. 8(4), 10.4172/jas.1000e138.

Current projects

SYNTHESIS AND ANTIENTEROVIRAL ACTIVITY OF NOVEL DIARRYL ETHERS AND THEIR COMPLEXIS WITH CYCLODEXTRINES

Project supervisor: G. Dobrikov, PhD, Institute of Organic Chemistry with Center for Phytochemistry, BAS

Project leader: A. S. Galabov, DSc

Project collaborators: Institute of Organic Chemistry with Center for Phytochemistry, BAS;

Research staff: V. Dimitrov, DSc; I. Nikolova, PhD; P. Grozdanov, PhD; A. Stoyanova, MS; L. Mukova, MS; N. Nikolova, MS

Compounds derivatives of the anti-enteroviral substance MDL-860, were synthesized in IOC-CPC – BAS. The synthesized 73 substances were tested against 3 enteroviruses – polio 1 (Sabin), Cxsackie B1 and Coxsackie B3. Two derivatives manifested a pronounced activity vs. poliovirus 1 and Coxsackievirus B1. A study on their activity in experimental neurounfections with Coxsackievirus B1 was started.

Grant Б-02-11/14 of the Bugarian Science Fund, Ministry of Education and Science

HUGHLY EFFECTIVE CHEMOTHERAPY OF COXSACKIEVIRUS B3 INFECTION

Project supervisor: I. Nikolova, PhD

Project leader: A. Stoyanova, MS

The in vivo effect of the triple combination following CAA treatment scheme was determined in mice infected with Coxsackievirus B3, Woodruff and Nancy strains.

Grant 2016/2017, supported by "Programme for Carrier Development of Young Scientists", BAS

STUDY OF THE COMBINATION ACTION OF OSELTAMIVIR AND S-ADENOSYL-L-METHIONINE (SAM) ON OXIDATIVE DAMAGES IN EXPERIMENTAL INFLUENA VIRUS INFECTION

Project supervisor: M. Mileva, PhD

Project leader: A. Dimitrova, MD

The combination effect of the glutathione precursor S-adenosyl-L-methionine (SAM) and oseltamivir in experimental influenza virus A/Aichi/68 (H3N2) infection in mice was studied. Influenza virus caused oxidative damages was recorded. On the base of the degree of lung consolidation as well on lung index an optimal effect was established at the combination of oseltamivir 1.25 mg/kg + SAM 50/100 mg/kg.

Grant 2016/2017, supported by "Programme for Carrier Development of Young Scientists", BAS

References:

Dimitrova, A., Mileva, M., Krastev, D., Gegova, G., Galabov, A. S.

Therapeututic strategy for survival of mice infected with influenza virus by combination of S-adnosyl-L-methionine and oseltamivir. *Acta Microbiol. Bulg*. 2016, 32, 133-138.

GENETIC PROFILE OF THE POPULATION OF BULGARIAN LANDS DURING THE THRACIAN PERIOD: NEW PERSPECTIVES IN BULGARIAN HISTORY

Project leaders: A. S. Galabov, DSc; D. Toncheva, DSc

Project collaborators: Department of Medical Genetics, Medical University of Sofia; Department of Anthropogenetics, University of Florence

Research staff: D. Caramelli, PhD; S. Karachanak-Yankova, PhD, D. Nesheva, PhD; J. Jordanov, DSc

Genetic analysis of mDNA (by NGS, including) in samples of bones and teeths of 70 individuals from Thracians tumuli from the 3rd millennium AC established the European belonging of Thracians. Moreover, it was found a biggest remoteness of the Thracian mDNA from the contemporary Bulgarians'mDNA by comparing with the mDNA samples of the Protobulgarians (8-10 BC). Besides, the Thracien mDNA manifested a biggest proximity to mDNA of contemporary Bulgarians in comparison with contemporary Greeks. This is the first by its gender investigation on this topic.

Project was sponsored by the University of Florence and a private Bulgarian sponsor.

Other studies:

EFFECT OF SMALL INTERFERING RNAs ON ENTEROVIRAL REPLICATION

A marked antiviral effect of small interfering RNAs (siRNAs) and of double-stranded RNAs (dsRNAs) on the replication of Coxsackieviruses B1 and B3 *in vitro*, was established, when oligofectamine was used as transfection reagent. siRNAs and dsRNAs of 2A and 3C were active toward Coxsackievirus B1, and siRNAs and dsRNAs of 5' UTR, 2A, 3D и 3C vs. Coxsackievirus B3. Touch Down RT PCR was used for the first time for multiplying specific fragments of Coxsackievirus B1 and B3 genomes. Applying this method dsRNAs of conservative regions of the Coxsackievirus B genome were obtained. Effective silencing of selected genome regions of Coxsackie B viruses was attained by application of specific dsRNAs and overlapping siRNAs, thus inhibiting the Coxsackie B viruses replication and elimination of the possibility for development of drug-resistant mutants.

These investigations were included of the second PhD dissertation of Nikolay Petrov, a regular PhD student of the Stephan Angeloff Institute of Microbiology (Acad. Angel S. Galabov, DSc was scientific supervisor of this PhD fellowship).

Reference

Petrov, N. M., Galabov, A. S. Small interfering RNAs against human enteroviral infections. *Acta Microbiol. Bulg*. 2016, 32, 101-107.

AROMATIC PRODUCTS OF BULGARIAN *ROSA ALBA* L.

Project supervisor: Milka Mileva, PhD

Aromatic products of Bulgarian *Rosa alba* L were studied for antiviral (against HSV1, HSV2, poliovirus 1 and Coxsackievirus B1), cytostatic and anti-mycotic effects, as well as antioxidant properties. This problematics was selected for a PhD fellowship.

References

Mileva, M. Oxidative stress as a target for medication of influenza virus infection. *Acta Micrbiol. Bulg.* 2016, 32 (3), 3-9.

Jovtchev, G., Stankov, A., Gateva, S., Georgieva, A., Dobreva, A., Dimiskovska, B., Mileva M. Does the essential oil from Rosa alba L. hide cytotoxic and genotoxic potential? Proceedings of the XX International ECO-Conference 2016 – Safe Food, 28-30th September 2016, Novi Sad, Serbia, COBISS, 2016, ISBN:978-86-83177-51-6, 209-216

EFFECTS OF ETHERIC OILS AND EXTRACTS OF BULGARIAN PLANTS

Effects of etheric oils and extracts of Bulgarian plants were investigated in Fuji cell line on the following markers: viability, intracellular level of oxygen active forms measured by DHE assay, OxySelect^{TM} ROS assay apoptosis activity test (analysis of expression of phosphatidylserine on the cellular surface) - Annexin V Detection и TAC Assay (analysis of the total antioxidant capacity of biological samples).

Grant of the National Institute for Radiologic Investigations (NIRS), Japan

DEPARTMENT OF IMMUNOLOGY

Final Summary

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

Project leader: A. Tchorbanov, PhD

Project collaborators: B.-L. Chiang, M. D., 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.

SCID mice humanization with patient's cells.

The immunomodulatory activity of Treg-of-B cells were tested in an *in vivo* experimental model of human SLE or human HDM allergy. Groups of six to eight week-old immunodeficient SCID mice were reconstituted by intraperitoneal (i.p.) injection of 10 to 15 x 10^6 cells in 500 µl of RPMI with PBMCs from clinically relevant SLE or HDM allergy patients. The control group of SCID mice was injected i.p. with PBMCs from healthy volunteers. The humanized mice were treated at weekly intervals and will be bled. The collected have been kept at - 70ºC.

The regulatory T cells induced by B cells have been generated by cultivation of

isolated B cells with OVA and $CD4^+$ $CD25^-$ T cells.

The changes of human cell populations after transfer in SCID mice with or without treatment were analyzed by flow cytometry. The kinetics of anti-DNA human IgG or anti-HDM human IgE antibodies as well as the cytokine secretion (IL-4, IL10, TNFα and IFN-γ) were determined by ultrasensitive ELISA. It was found the suppression of pathological antibodies production in both models after transfer of Tregs. Decreased levels of IL10, TNFα и IFN-γq but increase level of IL4 were also obtained in the group of treated animals.

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

Current projects

ADOPTIVE T CELL THERAPY AS A NEW APPROACH FOR PANCREATIC CANCER

Project leader: A. Tchorbanov, PhD

Project collaborators: Muobarak J. Tuorkey, PhD; Karolin K Abdul-Aziz, PhD; Nada S. Badr, PhD student; Riham Samir, Master student; Zoology Department, Faculty of Science, Damanhour Universityq Egypt

Research staff: N. Mihaylova, PhD; I. Manoylov, PhD student

Pancreatic cancer is one of the most lethal types of human malignancies, where infiltrating ductal adenocarcinoma of the pancreas (PDAC) has a poor prognosis. It accounts for over 85% of all pancreatic malignancies in which the patient has a short survival time. Despite the significant progress in the last few years and the better understanding for many molecular mechanisms underlying the development of PDAC, the progress in prevention, early diagnosis and treatment still needs major advances.

Indeed, pancreatitis hastens the process of metastasis, even in the early stages of the of pancreatic cells transformation to cancer cells. In line with this notion, the number of circulating pancreatic cells increases in mice following the induction of pancreatitis, where treatment with the anti-inflammatory drug, dexamethasone, blocked their numbers. A new approach for cancer treatment has been recently investigated, in which, T cells could be isolated from tumor, expanded in number in culture then re-infused into the patient. And or T cells are collected from peripheral blood, gene-modified to express a tumor targeting receptor, and then re-infused at large number into the patient. Whilst, these early reports of clinical responses are encouraging, similar work on tumors like pancreatic ductal adenocarcinoma has been less impressive.

Our hypothesis is that the lack of activity of T cell immunotherapy in these tumors is due to an over-representation of T cells that lack optimal anti-tumor activity.

We explore basic mouse model of pancreatic cancer. Tumors were initiated by s.c. injection of Balb/c animals with C-26 cell line ($5x10^5$ per mouse), and when tumors achieve progression, tumors were collected, disaggregated and the infiltrating T cell populations analyzed by multi-colour flow cytometric analysis for major T cell subsets (CD4, CD8), and activation markers CD25/CD69.

The isolated T cells were expanded in culture and then re-analysed for T cell subset and differentiation status. These T cells were tested for their ability to response against the relevant tumor target cell line *in vitro* by cytotoxic test and for their ability to

challenge the growth of short term (24 hour) or long term (7 day) established tumors in the Balb/c mouse model. These responses form the baseline against which subsequent manipulations will be tested.

Bilateral grant between Bulgarian Academy of Sciences and Academy of Scientific Research and Technology, Scientific & Cultural Relations Sector, Egypt

A NOVEL APPROACH FOR CANCER TREATMENT BY ACTIVATED MONOCYTE-DERIVED MACROPHAGES

Project leader: A. Tchorbanov, PhD

Project collaborators: F. Mariani, PhD; G. Cappelli, PhD; D. Giovannini, post-doc; A. Basso, PhD; Institute of Cell Biology and Neurobiology, National Research Council, Italy

Research staff: N. Mihaylova, PhD; I. Manoylov, PhD student

Various immunotherapeutic approaches have been used for the treatment of cancer. Today the most common therapies for malignant diseases are radiotherapy and hemotherapy, although they have severe side effects, due mostly to their non specific action. Different types of cancer need the development of new specific anti-cancer agents targeting cancer cells only without side effects.

The function of tumour-associated macrophages (TAMs) in different types of cancer patients is multifaceted and the literature shows conflicting roles. Many groups tried to characterize the M1 and M2 macrophage populations within TAMs in different subtypes of tumors compared to non-tumour tissue to ascertain their possible inflammatory or immunoregulatory role in the tumor microenvironment.

The differentiation of monocyte-derived-macrophages (MDMs) from the whole blood of healthy donors allows to test the immunoregulatory properties of a selected group of Mediterranean Plant Extracts on MDMs, both alone or in co-culture with tumor cells. The antioxidant, antimicrobial and antiproliferative activities of many Mediterranean plants have been shown in different studies, and we want to extend this analysis to melanoma and glioblastoma Human tumors.

Experimental therapy with newly developed drugs in humans is limited by technical and ethical restrictions. Understanding the functional characteristics in human disease where animal models fail to adequately replicate the human condition is a challenging task. Investigating the clinical importance of pathological hallmarks of human melanoma and glioma as tumor invasion in surgical biopsies has been limited to descriptive studies. Studies in humanized mouse models can circumvent some of these limitations. Severe combined immunodeficient (SCID) mice with T and B immunodeficiency syndrome are a model that is suitable for transferring of human cells without rejection and are unable to mount an adaptive immune response. SCID mice are perfect recipients and reconstituted with human tumor cells develop a symptoms specific for human diseases.

1. Establishment of animal models of tumor diseases in humanized SCID mice.

Humanized glioblastoma model development. Immunodeficient SCID mice were used for this set of experiments for reconstitution of glioblastoma cancer cell lines. The following parameters were observed: appearance of solid tumors; rate of tumor growth; flow cytometric analysis of infiltrates of immune cells in the microenvironment of the tumors; survival of the transferred groups.

2. MDMs differentiation from a first group of five healthy donors whole blood and plants extract treatment of MDMs.

Five healthy donors whole blood buffy coats were obtained by the University Policlinic Umberto I Transfusion Centre, and subjected to Ficoll gradient to separate

Peripheral Blood mononuclear cells (PBMCs). PBMCs were then kept in culture to obtain monocyte-derived-macrophages (MDMs).

Each of the five MDMs culture was treated with *Cinnamon zeylanicum* alcoholic extracts and their M1-M2 phenotype was analysed.

Bilateral grant between Bulgarian Academy of Sciences and CNR, Italy

RATIONAL DESIGN OF ANTIBODY REPERTOIRE PROBES USING PEPTIDE ARRAYS

Project leader: A. D. Pashov, MD, PhD

Project collaborators: E. Hovig, PhD; T. Clancy, PhD; Oslo University Hospital, Oslo, Norway

Research staff: Tch. Vassilev, MD, DSc; M. Hadzhieva, PhD student; V. Shivarov, postdoc; V. Kostov, student

During the second year of the main experiment of Deep Panning was performed and the work on the project was finished and reported. A random 7-mer peptide phage display library was panned on pooled IgM from 10 000 donors and then adsorbed on IgM myeloma. The selected phage were amplified and the peptide inserts deep sequenced. Out of 668 000 reads of high quality a subset of 224 000 reads with more than 2 counts per million were selected for further analysis. These were clustered by Gibbs Clustering algorithm and were found to cluster optimally in 790 sequence profiles providing a catalogue of typical molecular contexts of 7-mer IgM mimotopes. The optimal probing of the conformational space of IgM mimotopes, thus, could be achieved by taking one or few typical sequences from each cluster. Due to size limitations the clusters were further ranked by statistical significance and the best representative of the top 584 were further studied as a prototype library of IgM mimotopes representing optimally the repertoire in a limited set of peptides. Using sera from patients with glioblastoma multiforme, lung cancer brain metastases or non-tumor bearing surgery patients this library was compared to random peptides, random peptides purged of sequences significantly related to any one of the 790 profiles, to epitopes from tumor antigens, to theoretically predicted sequence (based on the cluster profiles found) and to 10 of the individual clusters of sequences. It was found that the proposed library had the highest reactivity with patients' IgM and the lowest mean correlation between the different peptide profiles across patients which proves that it is optimal. Interestingly the purged random library had very low reactivity proving that the 790 sequence profiles cover the majority of IgM epitopes (which are in the order of >10^6). The predicted sequences had as good or even better reactivity to the mimotopes found.

Thus, the deep panning strategy coupled to Gibbs Clustering was found to produce optimal mimotope libraries for the study of the IgM repertoire.

Grant BG09 D03-103 funded by the Financial Mechanism of the European Economic Area, Projects for Inter-Institutional Cooperation Measure

References:

Pashova, S., Schneider, C., von Gunten, S., Pashov, A. Antibody repertoire profiling with mimotope arrays. *Hum. Vaccin Immunother.* 2016 (in press).

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

Project leader: Tch. Vassilev, MD, DSc

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

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 polyspecificicty 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 polypecificity acqure novel biological properties and therapeutic potential, however these depend on the mechanisms for modification used.

Grant DFNI-B01-29 of the Bugarian Science Fund, Ministry of Education and Science

References:

Hadzhieva, M., Vassilev, T., Bayry, J., Kaveri, S., Lacroix-Desmazes, S., Dimitrov, J. D. Relationship between natural and heme-mediated antibody polyreactivity. *Biochem. Biophys. Res. Commun*. 2016, 472, 2061-2066.

New projects

SUPPRESSION OF AUTOREACTIVE B AND T CELLS BY PROTEIN CHIMERIC MOLECULES IN HUMANIZED SCID MODELS OF AUTOIMMUNE DIABETES MELLITUS

Project leader: A. Tchorbanov, PhD

Research staff: N. Mihaylova, PhD; I. Manoylov, PhD student; Silviya Bradyanova, PhD student; Gabrieala Boneva, student

Grant of the Bugarian Science Fund, Ministry of Education and Science

ANALYSIS OF NEW PHYSIOLOGICALLY ACTIVE BINDING SITES OF HUMAN C1q

Project leader: I. Tsacheva, PhD

Project leader for IM-BAS: A. Tchorbanov, PhD

Research staff: N. Mihaylova, PhD; I. Manoylov, PhD student; Silviya Bradyanova, PhD student

Grant of the Bugarian Science Fund, Ministry of Education and Science

SUPPRESSION OF THE FUNCTIONAL ACTIVITY OF COMPLEMENT SYSTEM AS A PREVENTIVE AND PROGNOSTIC APPROACH IN EXPERIMENTAL RHEUMATOID ARTHRITIS

Project leader: V. Gyurkovska, PhD

Research staff: L. Belenska-Todorova, PhD, P. Ganova, PhD student

Grant DM 03/4/17.12.2016 of the Bugarian Science Fund, Ministry of Education and Science

SECTION OF MYCOLOGY

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; N. Kostadinova, PhD; J. Miteva-Staleva, PhD student; S. Vassilev, MS; B. Spassova, MS; V. Dishliiska, MS; E. Evtova.

The ability of the white-rot fungi to degrade lignocellulosic materials is due to their highly efficient enzymatic system. They have extracellular enzymatic systems are responsible for polysaccharide degradation and a unique oxidative and extracellular ligninolytic system, which degrades lignin and opens phenyl rings. In addition, white rot fungi produce structurally diverse polyphenolic secondary metabolites which are known to responsible for the renowned antioxidative system of this fungal category. This study was focused on the evaluation of antioxidant properties of the 4 fungal strains belonging to the phylum *Basidiomycota*. They were identified as *Daedalea quercina, Fistulina hepatica, Lenzites warnieri*, and *Schizophyllum commune*. All of them possessed superoxide dismutase (SOD) and catalase (CAT) activity and the maximum was recorded after 72 and 96 h of cultivation, respectively. The total antioxidant activity of the culture fltrates determined by the DPPH method was strain-dependent and varied from 22 to 79% scavenging effect. Generally, the best results were achieved by D. quercina and L. warnieri. To our knowledge, these are the frst reports about antioxidant activity of *L. warnieri*.

Italian-Bulgarian bilateral project (2014-2018)

References:

Savino E., Girometta C., Miteva-Staleva J., Kostadinova A., Krumova E. Wood decay macrofungi: Strain collection and studies about antioxidant properties. *C. R. Acad. Bulg. Sci.* 2016, 69, 6, 747-755.

INNOVATIVE APPROACH FOR BIOLOGICAL TREATMENT OF LIGNOCELLULOSIC WASTES FOR BIOGAS PRODUCTION

Project supervisor: E. Krumova, PhD

***Project leader*:** N. Kostadinova, PhD

White-rot fungi of the Phylum *Basidiomycota* are quite promising in ligninolytic enzyme production and the optimization of their synthesis is of particular significance. The aim of this study was to investigate the effect of enhanced concentration of copper (Cu**)** ions (25 – 1000 μg/ml) on the activity of the ligninolytic enzyme complex (laccase, Lac; lignin peroxidase, LiP; Mn-peroxidase, MnP) in *Trametes trogii* 4_6, as well as the changes in the antioxidant cell response. All concentrations tested reduced significantly growth and glucose consumption. Cu ions

affected the ligninolytic enzyme activity in a dose dependent manner. Concentrations in the range of 25-100 μg/ml strongly stimulated Lac production (a 5-6-fold increase compared to the control). LiP activity was also induced by Cu, with the peak value being recorded following exposure to 50 μg/ml metal ions. In contrast, the addition of Cu ions had a positive effect on MnP activity at a concentration higher than 100 μg/ml. The maximum enzyme level was achieved at 1000 μg/ml. The results obtained on superoxide dismutase and catalase activities indicated that exposure of *T. trogii*46 mycelia to Cu ions promoted oxidative stress. Both enzyme activities were co-ordinately produced with Lac and LiP but not coordinately with MnP.

Grant DFNP-57/2016, supported by "Programme for Carrier Development of Young Scientists", BAS

References:

Kostadinova, N., Krumova, E., Boeva, R., Abrashev, R., Miteva-Staleva, J., Spassova, B., Angelova, M. (2016). Effect of copper ions on the ligninolytic enzyme complex and the antioxidant enzyme activity in the white-rot fungus *Trametes trogii* 4_6. *Plant Biosyst.* (in press).

EVALUATION OF THE GENETIC IDENTITY OF TWO FUNGAL STRAINS BELONGING TO SPECIES *TRICHODERMA LONGIBRACHIATUM*

Project leaders: M. Angelova, DSc; E. Krumova, PhD

***Project staff*:** R. Abrashev, PhD; N. Kostadinova, PhD; G. Stoyancheva, PhD; J. Miteva-Staleva, PhD student; B. Spassova, MS.

In connection with the contract signed between The Stephan Angeloff Institute of Microbiology, BAS, and the company "Valenza Biotech" Ltd., a research team from the Section of Mycology conducted experiments for comparison between two fungal strains by the following aproaches:

1. Molecular typing using PCR analysis of ITS1 и ITS4 regions
2. Evaluation of the translation elongation factor 1α (ITS rDNA)

Based on the studies carried out in accordance with the morphological and molecular methods, the both strains were identified as *Trichoderma longibrachiatum* (99% sequence identity with the data about *Trichoderma longibrachiatum* from DNA database BLAST). They showed the following identity: 100% **(**18S rRNA gene), 99% **(**Ttranslation Elongation Factor 1 alpha (tef1) gene), 99% **(**ITS- 18S ribosomal RNA gene- partial sequence) identity.

The research was financed by the company "Valenza Biotech" Ltd.,

New projects

CATALASE IN ANTARCTIC FUNGI: ANTIOXIDANT ROLE, LOCALIZATION, REGULATION AND PROPERTIES

***Project leader*:** E. Krumova, PhD

Project collaborator: S. Tosi, PhD, University of Pavia, Italy; G. Feller, PhD, University of Liège, Belgium

Research staff: M. Angelova, DSc; R. Abrashev, PhD; S. Stoitsova, PhD; N. Kostadinova, PhD; J. Miteva-Staleva, PhD student; G. Stoyancheva, PhD; P. Grozdanov, PhD; Ts. Paunova-Krusteva, PhD; I. Djumerska, PhD; B. Spassova, MS; D. Borissova, MS

Grant DN-01/1 of the Bugarian Science Fund, Ministry of Education and Science

ROLE OF AMARYLLIDACEAE ALKALOIDS IN THE ENDOPHYTE-HOST RELATIONSHIPS

Project supervisor: S. Berkov, Instute of Biodiversity and Ecosystem Research

Project leader: E. Krumova, PhD

Project staff: M. Angelova, DSc; N. Kostadinova, PhD; J. Miteva-Staleva, PhD student

Grant DN01/13 of the Bugarian Science Fund, Ministry of Education and Science

NEW NATURAL PEPTIDES WITH ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY, ISOLATED FROM SNAIL *CORNU ASPERSUM*

Project supervisor: P. Dolashka, Institute of Organic Chemistry

Project leader: M. Angelova, DSc

Project staff: R. Abrashev, PhD; N. Kostadinova, PhD; B. Spassova, MS.

Grant DN01/14 of the Bugarian Science Fund, Ministry of Education and Science

RESEARCH ON THE PRESENCE OF FILAMENTOUS FUNGUS IN SAMPLES ISOLATED FROM THE MAGURA CAVE

Project leaders: M. Angelova, DSc; E. Krumova, PhD

***Project staff*:** R. Abrashev, PhD; N. Kostadinova, PhD; G. Stoyancheva, PhD; J. Miteva-Staleva, PhD; B. Spassova, MS

Grant RD 11-00252 of of the Ministry of Culture, Republic of Bulgaria

LIST OF PUBLICATIONS NOT INCLUDED IN THE PROJECTS

Abrashev R., Feller G., Kostadinova N., Krumova E., Alexieva Z.**,** Gerginova M., Spasova B., Miteva-Staleva J.,Vassilev S., Angelova M. Production, purification, and characterization of a novel cold-active superoxide dismutase from the Antarctic strain *Aspergillus glaucus* 363. *Fungal Biol.* 2016, 120, 5, 679-689.

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Teneva-Angelova T., Hristova I., Pavlov A., Beshkova D. Lactic acid bacteria – from nature through food to health. In: Handbook of Food Bioengineering, Vol. 14, Progress of

Biotechnology in Food Industry, A. M. Holban and A. M. Grumezescu (Eds.). Elsevier (in press).

EDUCATION ACTIVITY

Lectures and practical Exercise

Acad. A. S. Galabov

Course in *Medical Virology*, St. Kliment Ohridski University of Sofia, Faculty of Biology
Course in *Antiviral Therapy*, University of Sofia "St. Kliment Ohridski", Faculty of Biology
Course in *Virology*, New Bulgarian University

Corr. Member H. Najdenski, DVMS

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 *Bioactive Substances from Cell Cultures*, University of Food Technologies, Plovdiv
Practical exercises in *Bioactive Substances from Cell Cultures*, University of Food Technologies, Plovdiv
Course in *Biotechnological Productions Based on Plant and Cell Cultures*, Agricultural University, Plovdiv
Practical exercises in *Biotechnological Productions Based on Plant and Cell Cultures*, Agricultural University, Plovdiv
Course in *Modern Technologies and Methods for Analysis of Bioactive Substances Chemistry*, University of Food Technologies, Plovdiv
Practical exercises in *Modern Technologies and Methods for Analysis of Bioactive Substances Chemistry*, University of Food Technologies, Plovdiv

Prof. M. Angelova, DSc

Course in *Oxidative Stress*, New Bulgarian University
Course in *Mycology*, New Bulgarian University

Assoc. Prof. S. Danova, DSc

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*, University of Sofia "St. Kliment Ohridski", Faculty of Biology
Practical exercises in *Methods of Electron Microscopy, Histochemistry and Immunocytochemistry in Cell Biology*, University of Sofia "St. Kliment Ohridski", Faculty of Biology

Assoc. Prof. A. Tchorbanov, PhD

Course in *Immunology*, New Bulgarian University
Practical exercises in *Immunology*, New Bulgarian University
Course in *Genetics*, University of Mining and Geology "St. Ivan Rilski

Assoc. Prof. A. Pashov, PhD

Course in *Tumor Cell/Tumor Immunology*, University of Sofia "St. Kliment Ohridski", Faculty of Biology

Assoc. Prof. D. Beshkova, PhD

Course in *Microbial Safety of Animal Products*, University of Food Technologies, Plovdiv

Course in *Microbiology of Fermented Functional Foods*, University of Food Technologies, Plovdiv

Assoc. Prof. E. Krumova, PhD

Practical exercises in *Oxidative Stress*, New Bulgarian University

Practical exercises in Mycology, New Bulgarian University

Practical exercises in *Cytology, Hyistology and Embryology*, University of Sofia "St. Kliment Ohridski", Faculty of Biology.

Assoc. Prof. M. Mileva, PhD

Course in *Pharmacology of Eye Diseases*, University of Sofia "St. Kliment Ohridski", Faculty of Physics

Course in *Hygiene and Health Education*, University of Sofia "St. Kliment Ohridski", Faculty of Pedagogic

Assist. Prof. M. Stoilova-Disseva, PhD

Practical exercises in *Genetics*, University of Sofia "St. Kliment Ohridski", Faculty of Biology

Assist. Prof. Ivanka Nikolova, PhD

Course in *Virology*, New Bulgarian University

Course in *Bacterial and Viral Infections of Eye*, University of Sofia "St. Kliment Ohridski", Faculty of Physics

Practical exercises in *Virology*, New Bulgarian University

Assist. Prof. Ts. Paunova-Krasteva, PhD

Practical exercises in *Cell Biology and Pathology*, University of Sofia "St. Kliment Ohridski", Faculty of Biology

Assist. Prof. L. Simeonova, PhD

Practical exercises in *Virology*, University of Sofia "St. Kliment Ohridski", Faculty of Biology

Assis. Prof. N. Kostadinova, PhD

Practical exercises in *Cytology and Cell Biology,* University of Sofia "St. Kliment Ohridski", Faculty of Biology

Assist. Prof. S. Rusinova-Videva, PhD

Practical exercise in *Micology*, Plovdiv University Paisii Hilendarski, Plovdiv

Practical exercise in *Hydrobiology*, Plovdiv University Paisii Hilendarski, Plovdiv

Practical exercise in *Hydrobiology*, Plovdiv University Paisii Hilendarski, Branch Smolyan

Assis. Prof. A. Kroumov, PhD
Course in *Photobioreactors-modeling, optimization, design and scale up*, (for Master, PhD students and professors) West Parana State University, Toledo, Parana, Brazil

PhD Students

Boteva N. *Diversity and biotechnological potential of Archaea from Bulgarian hot springs*.
Advisor: Assoc. Prof. M. Kambourova, DSc

Bradyanova S. *Functional elimination of autoreactive T cells by antibody therapy in murine and Humanized SCID models of systemic lupus erythematosus.*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Dimitrova L. *New antimicrobial agents with therapeutic potential*
Advisors: Corr. Member H. Najdenski, DSC and Corr. Member V. Bankova, DSc

Dimitrova, A. *Study of the combined action of S-adenosyl-L-methionine and oseltamivir on the pathoegenis of experimental influenza virus infection in mice*
Advisors: Acad. A. S. Galabov, DSc and Assoc. Prof. M. Mileva, PhD;
Research Consultant: Assoc. Prof. D. Krastev, PhD

Ganova P. *Influence of the kinase inhibitor berberine on the processes of osteoclstogenesis 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 G. *Comparative studies on the specificity and biological potential of different batches of tuberculin*
Advisor: Assoc. Prof. M. Bonovska, PhD

Haloglu P. *In vitro cultures of Fabiana imbricata Ruiz. et Pav. as a technological matrixes for bioactive substances production*
Advisors: Prof. A. Pavlov, DSc and Assoc. Prof. S. Yahcheva, PhD

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.*
Advisor: Assist. Prof. A. Kroumov

Ivanova I. *Antigen targeting by genetically engineered chimeric molecules*
Advisor: Assoc. Prof. A. Tchorbanov, 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 fun*gi
Advisor: Prof. M. Angelova, DSc

Nemska V. *Biotechnological Characteristics of Lactic Acid Bacteria.*
Advisor: Assoc. Prof. S. Danova, DSc

Stoyanova A. *Highly effective combination of anti-enteroviral inhibitors at experimental newronfections with Coxsackievirus B1*
Adviser: Acad. A. S. Galabov, DSc

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

Teneva-Angelova T. *Biodiversity of lactic acid bacteria in medicinal plants and possibilities for exploitation of their biological potential*.
Advisor: Assoc. Prof. D. M. Beshkova, PhD

Tzvetanova K. *Microbial Biodegradation of Industrial Pollutants of the Environment.*
Advisor: Assoc. Prof. Z. Alexieva

Velikova P. *Polysaccharide-modifying lactic acid bacteria for use in the food industry.*
Advisor: Assoc. Prof. P. Petrova

Yazova D. *Agrobacterium rhizogenes-mediated genetic transformation of plants from the genus Verbascum and metabolic analyses*
Advisors: Assoc. Prof. M. I. Georgiev, PhD andAssist. Prof. A. Marchev, PhD

Defended PhD thesis

Petrov, N. *Effect of small interfering RNAs on enteroviral replication*
Advisor: Acad. A. S. Galabov, DSc

Defended MS Diploma Thesis

Bibova R. *Microbial biodegradation of phenol and phenol derivatives by fungal strains isolated from Antarctic soils.*
Advisor: Assoc. Prof. Z. Alexieva

Bradyanova S. *Autoreactive T cell neutralization by therapy with monoclonal antibody in pristane-induced systemic lupus erythematosus.*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Chipinski P. *Immune therapeutic properties of anti-Annexin A1 in humanized mouse model of lupus.*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Hlebarska D. *Recombinant immune-toxin construction for targeting of multiple myeloma cell marker.*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

Milev N. *Creation of new synbiotic yeasts featuring Pediococcus acidilactici and Lactobacillus paracasei*
Advisor: Assoc. Prof. P. Petrova, PhD

Nikolova B. *Biotechnological significance and biological activity of lactic acid bacteria.*
Advisor: Assoc. Prof. S. Danova, DSc

Nikolova, L. A. Combination effects of 2-(3,4-dinitrobenzonitryl) (MDL-860), guanidine hydrochloride and 2-(α-hydroxibenzyl)-benzimidazole (hbb) against poliovirus type 1 and Coxsackievirus B3
Adviser: Acad. A. S. Galabov, DSc
Research Consultant: A. Stoyanova, MS

Defended BS Diploma Thesis

Amerikova M. *Evaluation of chemical stability and biological activity of alpha-defensin type 2 antimicrobial peptides.*
Advisors: Corr. Member H. Najdenski, DSc and Prof. V. Pencheva, PhD (Faculty of Pharmacy, Medical University, Sofia)

Boneva G. *Generation of protein chimeric molecules for specific suppression of pathological B cells in model of autoimmune diabetes.*
Advisor: Assoc. Prof. A. Tchorbanov, PhD

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

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

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

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

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

Kumanova E. *Experimental immunology*.
Advisor: Assoc. Prof. A. Tchorbanov, PhD

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

Davidkova V. *Experimental immunology*.
Advisor: Assoc. Prof. A. Tchorbanov, PhD

CELEBRATION DAY OF THE STEPHAN ANGELOFF INSTITUTE OF MICROBIOLOGY

The Day of Institute, March 14, was celebrated with a ceremonial meeting. The meeting already turned into tradition, 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. Athanassios Tsakris**,** PhD, MD from University of Athens, Athens, Greece that gave a very intriguing lecture entitled "Antimicrobial resistance as a global threat in times of economic difficulties: the paradigm of carbapenemase-producing pathogens".



Acad. Angel S. Galabov, delivered the traditional Annual Prize for the best scientific publication by young microbiologist (under 35 year of age) to Adelina Stoyanova, MS (The Stephan Angeloff Institute of Microbiology, Department of Virology) and Maya Hadzhieva, PhD (The Stephan Angeloff Institute of Microbiology, Department of Immunology).

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:

Maya Hadzhieva, PhD (The Stephan Angeloff Institute of Microbiology, Department of Immunology) for the following paper: Hadzhieva M., Vassilev T. L˙, Roumenina L. T., Bayry J., Kaveri S. V. (2015). Lacroix-Desmazes S**.**, Dimitrov J.D. Mechanism and functional implications of the heme-induced binding promiscuity of IgE. *Biochemistry*, 54(11): 2061-2072.

Adelina Stoyanova, PhD student (The Stephan Angeloff Institute of Microbiology, Department of Virology) for the following paper: Stoyanova A., Nikolova I., Galabov A. S. (2015). Effect of consecutive alternating administration (CAA) of a triple anti-enteroviral combination on Coxsackievirus B1 neuroinfection in mice. Antiviral Res.;121:138-44.


WORKSHOP "FOOD-BORNE PATHOGENS AND FOOD SAFETY", PRE-CONFERENCE MEETING FOR THE INTERNATIONAL CONFERENCE ON FOOD MICROBIOLOGY (BIRMINDGHAM, U

During 26-27 May, 2016, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, in co-operation with OMICS International Conferences, was the host of the workshop "Food-Borne Pathogens and Food Safety", a pre-conference meeting for the International Conference on Food Microbiology (Birmingham, UK, August 08-10. 2016). The meeting focused on the epidemiology and ecology of food-borne diseases, methods for evaluation and prevention of food contamination with microbial pathogens, as well as estimation of the risks related with food safety. The meeting programe included 8 plenary lectures, 5 oral presentations and 15 posters, with a total list of 96 authors from Bulgaria and other Balkan countries. The Bulgarian participants in the workshop were lecturers and students from the Institute of Microbiology and Institute of Experimental Morpho;ogy, Pathology and Antropology with Museum, Bulgarian Academy of Sciences, National Diagnostic and Research Veterinary Institute „Prof. G. Pavlov", Sofia University "St. Kliment Ohridski", Thracian University, Stara Zagora, Medical University - Sofia, Plovdiv University, and New Bulgarian University. Foreign participants came from the Public Health Institute of Belgrade, Serbia, and the Institute of Public Health of Republic Macedonia, Skopje. The meeting participants agreed on the importance of coordinating the scientific potential and the research efforts within joint scientific projects in the field of food safety.

The meeting was organized by Prof. Hristo Najdenski, DVM, DSc, Assoc. Prof. Stoyanka Stoitsova, PhD, Assistant Prof. Tsvetelina Paunova-Krasteva, PhD, Assistant Prof. Dayana Borisova (members of Organising Committee).




INTERNATIONAL CONFERENCE "DIVERSITY AND BIOTECHNOLOGICAL POTENTIAL OF EXTREMOPHILES"

Laboratory Extremophilic Bacteria organized an International conference "Diversity and biotechnological potential of extremophiles on September 26th and 27th 2016" as a part of the performance of the project D03-100 "Biodiversity and biotechnological potential of Archaea from Bulgarian hot springs", funded by Financial Mechanism of the European Economic Area, "Projects for inter-institutional cooperation" measure and The Staphan Angrloff Institute of Microbiology. Leading scientists from eight European countries (Germany, England, Norway, Italy, Russia, Sweden, Armenia and Romania) were invited as lecturers. This conference was the first meeting caried out in Bulgaria with participation of scientists working in the field of extremophilicity. It gave new opportunity for Bulgarian scientists to meet world famous scientists working in the field and provided an options for future collaborations with European teams.

SEMINAR ON GENOMICS METHODS IN TUMOR IMMUNOLOGY

A seminar on Genomics Methods in Tumor Immunology was organized by Assoc. Prof. Anastas Pashov, SAIM, BAS, Sofia, on September 26, 2016 in the frame the European Economic Area (EEA) and funded by Financial Mechanism of the EEA, "Projects for inter-institutional cooperation" measure and The Staphan Angrloff Institute of Microbiology. Participants: Prof. Eivind Hovig (Olso University Hospital), Prof. Vessela Kristensen (Oslo University Hospital) etc

3rd BLACK SEA INTERNATIONAL IMMUNOLOGY SCHOOL (BSIIS2016)

3rd Black Sea International Immunology School was held on 14-16 October 2016 in Hotel "Diplomat Plaza" - Lukovit, Bulgaria. The school was funded by EFIS. Organizer: Assoc. Prof. A. Tchorbanov, PhD - President of the Bulgarian Society for Immunology (BuSI)

FOURTH NATIONAL CONGRESS OF VIRILOGY WITH INTERNATIONAL PARTICIPATION/ DAYS OF VIROLOGY IN BULGARIA

The event was organized by the Bulgarian Society for Microbiology at the Union of Scientists in Bulgaria, Sofia, May 18-20, 2016. This major scientific forum was chaired by

Academician Angel S. Galabov. Very active participation was manifested by virologists from the Department of Virology, the Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sceiences. A total of 50 research papers (including eight plenary) were presented, devoted to the latest advances in the field of medical, veterinary and plant virology. Eminent scientists as Prof. Francis Delpeyroux from Institut Pasteur (Paris), Prof. Istvan Tobias (Budapest), Prof. Anna Papa (Thessaloniki), Prof. Rajarshi Gaur from India, attended the congress. Bulgarian virologists were represented by the teams of Acad. Angel S. Galabov and Prof. Tatayana Chervenyakova (Clinics of Infectious Diseases, Medical University of Sofia), Asoc. Prof. Lilia Ivanova (Medical Univesrsity of Varna), Assoc. Prof. Stoyan Shishkov (Faculty of Biology, St. Kliment Ohridski, Sofia University), Assoc. Prof. Anthony Stoev and Assoc. Prof. Bistra Dikova (Pushkarov Institute), Prof. Radka Argirova (Tokuda Hospital), Prof. Raiko Peshev (National Diagnostic and Research Veterinary Institute) , and many others. The scientific forum ended with a round-table discussion on “Training in Virology at Uiversities”.

Awards for 2016

L'Oréal-UNESCO Prize "For Women in Science"

L'Oréal-UNESCO Prize "For Women in Science" for 2016 was receved by Kalina Nikolava-Ganeva, PhD (Department of Immunology).

Awards of Congress and Conferences

First prize for presentation on 3rd Black sea International Immunology School (BSIIS2016, 14-16 October 2016, Hotel "Diplomat Plaza" - Lukovit, Bulgaria) was received by Iliyan Manoilov, PhD student (Department of Immunology).

First Prize for poster presentation on Jubilee Scientific Conference „10 years Bulgarian Association of Clinical Immunology", 28-29 October, 2016 – Sofia Hotel Balkan, Sofia, Bulgaria was received by Gabriela Boneva (Department of Immunology), received the.

Prize for a best poster presentation on Workshop on food-borne pathogens and food safety, 26.05.2016 - 27.05.2016, Sofia, Bulgaria - Pre-Conference Meeting for International Conference on Food Microbiology, Birmingham, UK, August 08-10, 2016 was received by Maya Zaharieva (Departament of Infectious Microbiology).

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