# TWELFTH CONGRESS
# OF THE BULGARIAN MICROBIOLOGISTS

## with International Participation

## PROGRAM AND ABSTRACTS

Yundola, Bulgaria, October 11-14, 2010

# PROGRAM AND ABSTRACTS

## Twelfht Congress
## of the Bulgarian Microbiologists
## with International Participation

**Yundola, Bulgaria**
www.micb-congress12.com

October 11-15, 2010

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## CONGRESS ORGANIZERS

Union of Scientists in Bulgaria –
Bulgarian Society for Microbiology

with associated

Bulgarian Society of Medical Microbiology
Bulgarian Society of Medical Virology
Bulgarian Society of Plant Virology "Prof. Dr. D. Atanasoff"

and

The Stephan Angeloff Institute of Microbiology,
Bulgarian Academy of Sciences

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## CONTRIBUTOR

The Stephan Angeloff Institute of Microbiology

Bulgarian Academy of Sciences

## CONGRESS SECTIONS

General and Applied Microbiology (GAM)

Medical Microbiology (MM)

Veterinary Microbiology (VM)

Virology (V)

Infectious Immunology (II)

Plant and Soil Microbiology (PSM)

## SOCIAL PROGRAM

**Monday, October 11**
**19:30** Concert
**20:00** Welcome Dinner

**Tuesday, October 12**
**19:00** Congress Banquet

**Thursday, October 14**
**09:00** Sightseeing Tour

# FINAL PROGRAM

**Registration:** Monday, October 11 12:00 – 18:00
Tuesday, October 12 08:30 – 18:00

***Monday, October 11***

**Opening Ceremony**

**Great Hall**

**Chairpersons:** *Angel S. Galabov*
*Hristo Najdenski*

**17:30** Welcoming Addresses
President of the Organizing Committee
Mayor of the City of Velingrad
Ministry of Health
Federation of European Society for Microbiology (FEMS)
Balkan Society of Microbiology

**18:15** Memoria
Academician Ignat Emanuilov
Professor Simeon Galabov
Corr. Member Kalcho Markov

19:00 FEMS activities and possibilities for FEMS grants, V. Talevski, FEMS Executive Committee Member, Grands Secretary

**19:30** Concert
**20:00** Welcome Dinner

# ORAL PRESENTATIONS

## Tuesday, October 12

### Hall 1: Virology

***Session I***

Chairpersons: A. S. Galabov, *Sofia*
A. Papa, *Thessaloniki*

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|---|---|---|
| **9:00** | **V1**<br>**Plenary** | ***Hristo Roussev* Memorial Lecture**<br>**Blue tong in ruminants: history of the diseases in Europe, problems and perspectives of prevention and control**<br>*G. Georgiev, Sofia* |
| **9:30** | **V2**<br>**Plenary** | **Hristo Roussev Memorial Lecture**<br>**Avian influenza virus: history of the diseases in Europe, H5N1 problems and perspectives of prevention and control**<br>*I. Zarkov, Stara Zagora.* |
| **10:00** | **V3**<br>**Plenary** | **Emerging viruses in Greece: Crimean Congo hemorrhagic fever virus in 2008 – West Nile virus in 2010**<br>*A. Papa, Thessaloniki* |
| **10:30** | | Break |
| **11:00** | **V4**<br>**Plenary** | **Different mechanisms of interactions between HIV and viruses causing oppotunustic and co-infections in HIV infected patient – an evident- based hypothesis**<br>*R. Argirova, I. Tsekov, R. Gavazova, K. Borisov, Z. Kalvachev, Sofia.* |

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| **11:30** | **V5** | **Dynamics of HIV-1 replication and infectivity in experimental co-infection "HIV-1 + human influenza (Flu) virus" – implication to pathogenesis in HIV-infected individuals**<br>*R. Gavazova, D. Ivanov, S. Raleva, P. Genova –Kalou, S. Pavlova, R. Kotseva, R. Argirova, Sofia.* |
| **11:45** | **V6** | **Approaches for the enhancement of the antienteroviral effect of oxoglaucine**<br>*L. Nikolaeva-Glomb, A. Stoyanova, A. S. Galabov, Sofia.* |
| **12:00** | **V7** | **Anti-HIV activity of a newly synthesized ester of abacavir – A-Gly**<br>*K. Stanoeva, A. Hinkov, P. Genova – Kalou, S. Raleva, I. Stankova, R. Chayrov, R. Argirova, Sofia.* |
| **12:15** | **V8** | **Two newly synthesized 2-styryl-8-hydroxyquinolines (8-SQs) derivatives with anti-HIV-1 activity targeting viral integrase and protease**<br>*A. Hinkov, K. Stanoeva, V. Atanasov, P. Genova –Kalou, S. Raleva, A. Pavlov, S. Chervenkov, R. Argirova, Sofia.* |
| **12:30** | **V9** | **Presence of Rare HIV-1 Genetic Forms: CRF 04_cpx, CRF 05_DF and CRF 36_cpx in Bulgaria**<br>*I. Alexiev, D. Beshkov, L. Karamacheva, V. Georgieva, I. Elenkov, Sofia.* |
| **12:45** | | ***Session end*** |

***Session II***

Chairpersons: R. Argirova, *Sofia*
I. Zarkov, *Stara Zagora*

**14:00** **V10** **IDflu: a new approach to the seasonal anti-flu vaccination**
*A. S. Galabov, Sofia*

**14:30** **V11 Plenary** **Antioxidants in prevention and therapy of influenza virus infection**
*M. Mileva, Sofia.*

**15:00** **V12** **Effect of a novel protease inhibitor from Streptomyces chromofuscus on lung injury in influenza virus-infected mice and its physico-chemical characteristics**
*J. Serkedjieva, M. Dalgalarrondo, L. Angelova-Duleva, I. Ivanova, Sofia, Nantes*

**15:15** **V13** **Phenotyping and genotyping characterization of resistance to M2 blockers and neuraminidase inhibitors of seasonal influenza virus strains isolated in Bulgaria during the period 2004 – 2007**
*L. Simeonova, L. Mukova, G. Gegova, P. Grozdanov, R. Kotseva, S. Van der Werf, A. S. Galabov, Sofia, Paris*

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| **15:30** | **V14** | **Synergistic effects of ellagitannins with acyclovir on the replication of herpes simplex virus type 2**<br>*N. Vilhelmova-Ilieva, S. Quideau, A. S. Galabov, Sofia, Nantes* |
| **15:45** | **V15** | **Antiherpes activity of some medical plants from Lamiaceae**<br>*S. Shishkov, D. Todorov, M. Dimitrova, K. Kostova, A. Atanasova, V.Kapchina-Toteva, Sofia.* |
| **16:00** | **V16** | **Application of real-time PCR in complex diagnostics of infections with Epstein-Barr virus**<br>*D. Velcheva, V. Tsoncheva M. Boyanova, Sofia* |
| **16:15** | | ***Session end*** |

***Session III***

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|---|---|---|
| Chairpersons: | | Kalvachev, *Sofia*<br>P. Teoharov, *Sofia* |
| **16:45** | **V17 Plenary** | **Novel human polyomaviruses – possible cancerogens?**<br>*Z. Kalvatchev, I. Tsekov, A. Kumanova, Sofia.* |
| **17:15** | **V18** | **Diagnostic difficulties when studying JC polyomavirus in tumor samples**<br>*I. Tsekov, A. Petrova, Z. Kalvatchev, Sofia.* |
| **17:30** | **V19** | **Detection and genotyping of human papilloma viruses in Bulgarian patients for the period of 2009-2010**<br>*P. Grozdanov, V. Zlatkov, G. Ganchev, I. Karagyozov, A. S. Galabov, Sofia.* |
| **17:45** | **V20 Plenary** | **Genetic diversity of hepatitis C virus among Bulgarian injecting drug users with hepatitis C**<br>*P. Teoharov, I. Pavlov, Sofia*. |
| **18:15** | **V21** | **Parenterally transmitted hepatitis viruses in clinically diagnosed cases of acute hepatitis and chronic lived diseases.**<br>*L. Ivanova, J. Stoykova, D. Tzaneva, Varna* |
| **18:30** | | ***Session end*** |

## Tuesday, October 12

### Hall 2: General and Applied Microbiology

***Session I***

Chairpersons: Z. Alexieva, Sofia
A. Krastanov, Plovdiv

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| **9:00** | **GAM1 Plenary** | **Compatible solutes in thermophiles and hyperthermophiles from the esoteric to the applied**<br>*M. S. Da Costa, N. Empadinhas, Coimbra* |
| **9:30** | **GAM2 Plenary** | **Gram negative lipopolysaccharide and peptidoglycan as elicitors of innate immunity system in mammals and plants**<br>*A. Molinaro, Napoli* |
| **10:00** | **GAM3 Plenary** | **Application of probiotic bacteria in functional foods**<br>*Z. Denkova, I. Murgov, Plovdiv* |
| **10:30** | **GAM4 Plenary** | **Metabolic alterations during growth of Lactobacillus strains in the presence of oligosaccharides and whey proteins**<br>*T. Mandadzhieva, Ts. Ignatova-Ivanova, I. Iliev, I. Ivanova Sofia, Shumen, Plovdiv* |
| **11:00** | | Break |
| **11:30** | **GAM5** | **Начини за транспорт на микробиологични проби за изследване и анализ**<br>*World Courier Bulgaria Ltd., Sofia* |

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| **11:50** | **GAM6** | **Масспектрометърът MALDI Biotyper (Bruker Daltonics, Germany) – микробиалната идентификация на XXI век**<br>*N. Bajnov, Medical Technics Engineering Ltd, Sofia* |
| **12:10** | **GAM7** | **Developments and achievements in microbial production of amino acids at the Institute of Microbiology - BAS**<br>*A. Ratkov, Sofia* |
| **12.30** | | Lunch |

***Session II***

Chairpersons: S. Groudev, *Sofia*
S. Stoitsova, *Sofia*

**14:00** **GAM8 Plenary** **Anaerobic digestion of organic wastes - state of the art and some perspectives**
*I. Simeonov, D. Denchev, Sofia*

**14:30** **GAM9** **Biotechnological production of galanthamine**
*I. Ivanov, V. Georgiev, M. Ilieva, S. Berkov, Atanas Pavlov, Plovdiv, Sofia*

**14:50** **GAM10** **Determination of capsular polysaccharides in two clinical isolates of Acinetobacter baumannii, smal and MG1**
*E. Fregolino, O. Holst, R. Lanzetta, M. Parrilli, C. De Castro, Napoli*

**15:10** **GAM11** **Biofilms of Escherichia coli: effects of secreted metabolites of other bacteria on growth and development**
*S. Stoitsova, A. Vacheva, S. Danova, R. Georgieva, R. Ivanova, S. Kostadinova, M. Marhova, K. Vasileva, T. Velinov, M. Bivolarska, Y. Baldjiska, Sofia, Plovdiv*

**15:30** **GAM12** **In vitro selection of lactobacilli with probiotic potential for development of new functional foods and therapeutic formulas**
*S. Danova, R. Georgieva, R. Tropcheva, J. Manassiev, J. Dermendjieva, N. Ivanovska, Sofia*

**15:50** Break

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| **16:30** | **GAM13** | **Biotechnological production of cold-active superoxide dismutase by antarctic fungus aspergilus Glaucus 363**<br>*V. Dishliyska, S. Pashova, L. Stefanova, K. Krumova, Z. Alexieva, H. Jemendzhiev, B. Spassova, S. Vassilev, M. Angelova, Sofia* |
| **16:45** | **GAM14** | **Biochemical and genetic studies on the production of neuraminidase by environmental isolates of v. cholerae non-o1 from Bulgaria**<br>*R. Eneva, S. Engibarov, T. Strateva, R. Abrashev, I. Abrashev, Sofia* |
| **17:00** | **GAM15** | **Cold adaptation and oxidative stress response of two filamentous fungi, isolated from Livingston island, Antarctica**<br>*N. Kostadinova, E. Krumova, S. Pashova, J.Miteva, M. Angelova, Sofia* |
| **17:15** | **GAM16** | **Molecular characterization of bacterial cultures isolated from xenobiotic polluted environment and its possible application in bioremediation technologies**<br>*G. Satchanska, Y. Topalova, S. Selenska-Pobell, R. Dimkov, E. Golovinsky, Sofia, Drezden* |
| **17:30** | | ***Session end*** |

## Wednesday, October 13

### Hall 1: Veterinary Microbiology

***Session I***

Chairpersons: H. Najdenski, *Sofia*
G. Georgiev, *Sofia*

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| **9:00** | **VM1 Plenary** | **Rapid methods for detection and quantification of food-borne pathogens – implications for risk assessment**<br>*H. Najdenski, Sofia* |
| **9:30** | **VM2 Plenary** | **Zoonotic diseases and the role of horses and other equids as its source**<br>*I. Chenchev, S. Chakarova, G. Georgiev, Sofia* |
| **10:00** | **VM3 Plenary** | **Sequencing analysis of glicoprotein G gene of some Bulgarian isolates of CapHV 1**<br>*I. Sirakov, R. Peshev, Sofia* |
| **10:30** | **VM4** | **Prevalence of tularemia among domestic animals in Sofia region**<br>*S. Ivanova, Sofia* |
| **10:45** | | ***Session end*** |

## Tuesday, October 12

### Hall 2: Plant and Soil Microbiology

***Session I***

Chairpersons N. Bakardjieva, *Kostinbrod*
A. Stoev, *Kostinbrod*

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| **14:00** | **PSM1 Plenary** | **Genetic diversity of Wheat dwarf virus in Europe**<br>*I. Tóbiás, N. Bakardjieva,O. Shevchenko, B. Kiss, A. Bysov and L. Palkovics, Budapest, Kostinbrod, Kyiv* |
| **14:30** | **PSM2** | **Tomato spotted wilt virus (TSWV) on medical plants in Bulgaria**<br>*B. Dikova, Kostinbrod* |
| **14:45** | **PSM3** | **Efficacy evaluation problems associated with the analysis of the risk for resistance to plant protection products in Bulgaria**<br>*A. Stoev, N. Bakardjieva and T. Vatchev, Kostinbrod* |
| **15:00** | | ***Discussion*** |

## Wednesday, October 13

### Hall 2: Medical Microbiology

***Session I***

Chairpersons: L. Boyanova, *Sofia*
I. Christova, *Sofia*

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| **9:00** | **MM1 Plenary** | **The most common disease of the nails - onychomycosis**<br>*S. Radulovich, Belgrade* |
| **9:30** | **MM2 Plenary** | **Evaluation of cagE and cagA genotypes in Helicobacter pylori strains from Bulgarian symptomatic patients**<br>*L. Boyanova, D. Yordanov, G. Gergova, R. Markovska, I. Mitov, Sofia* |
| **10:00** | **MM3** | **Association of vacA i alleles in Helicobacter pylori with the disease, place of residence and previous treatment of the patients**<br>*D. Yordanov, L. Boyanova, R. Markovska, G. Gergova, I. Mitov, Sofia* |
| **10:15** | **MM4** | **Modern diagnostic of tick-borne and some zoonotic infections in Bulgaria**<br>*I. Christova, Sofia* |
| **10:30** | | Break |
| **11:00** | **MM5** | **Mini-symposium of AA Medical Bulgaria - official representative of Bio-Rad**<br>**Alternative methods in food testing**<br>*Alexandra Livescu,*<br>*Product Manager Emerging Markets,*<br>*Bio-Rad* |

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| **11:30** | **MM6** | **Bio-Rad solution for clinical microbiology laboratories**<br>*Florina Toma, BVD and CMD Product Specialist, Bio-Rad* |
| **12:30** | **MM7** | **Bio-Rad solutions for water testing – Check 'N' Safe your bathing water**<br>*Alexandra Livescu, Product Manager Emerging Markets, Bio-Rad* |
| **13:00** | | Lunch |

***Session II***

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|---|---|---|
| Chairpersons: | | T. Kantardjiev, *Sofia*<br>S. Radulovich, *Belgrade* |
| **14:00** | **MM8** | **GlaxoSmithCline - symposium**<br>**European view on vaccine prophylaxis**<br>*T. Kantardjiev , Sofia* |
| **14:30** | **MM9** | **Biology and unique mechanisms of behavior in vivo and in vitro of Mycobacterial L-forms and their role in latent tuberculosis**<br>*N. Markova, Sofia* |
| **14:45** | **MM10** | **Antibiotic resistance characteristics and strain diversity in Acinetobacter baumanii**<br>*R. Vatcheva-Dobrevska, T.J.K. van der Reijden, E. van Strijen, E. Keuleyan, H. Hitkova, E. Savov, M. Lesseva, I. Ivanov, E. Dobreva, M. Grigorova, A. Dimitrova, V. Tolekova, T. Yovcheva, P.J. van den Broek, L. Dijkshoorn, Sofia, Pleven, Varna, Yambol, V. Tarnovo, Leiden* |
| **15:00** | | ***Session end*** |

# POSTER PRESENTATIONS

## Tuesday, October 12

**Great Hall: General and Applied Microbiology**

***Poster Session 1***
**16.30 – 18.00**

**GAM17** **Antimicrobial potential of selected thiosulfonates-based biocides and biosurfactants against bacteria and fungi**
*A. Sotirova, T. Avramova, I. Lazarkevich, V. Lubenets, E. Karpenko, D.Galabova, Sofia, Lviv*

**GAM18** **Biofilm-related phenotypes in Escherichia coli K-12: effects of late stationary phase supernatants**
*A. Vacheva, R. Ivanova, S. Stoitsova, Sofia*

**GAM19** **Two polysaccharide antigens associated with the surface of Escherichia coli o157:H- and their impact in iteractions with the host**
*S. Stoitsova, C. De Castro, A. Molinaro, E. Nikolova, R. Ivanova, V. Pavlova, Ts. Paunova., V. Kamburov, Sofia, Napoli*

**GAM20** **Superoxide dismutase enzymes respiratory yeasts Pichia pini**
*D. Koleva, V. Petrova, Zl. Uzunov, A. Kujumdzieva, Sofia, Napoli*

**GAM21** **Effects of extracts from bulgarian medicinal plants on biofilm formation by upec clinical isolates**
*S. Stoitsova, B. Mustafa, J. Staneva, M. Marhova, A. Vacheva, S. Kostadinova, M. Todorova, R. Ivanova, Sofia, Plovdiv*

**GAM22** **HeLa cells localization of the microtubule-related transport activator vdp upon interaction with E. coli o157:H-**
*V. Levakova, E. Marinchevska, T. Topuzova-Hristova, R. Ivanova, S. Stoitsova, Sofia*

**GAM23** **Calgevax (BCG) in treatment of recurrent respiratory papillomatosis**
*T. Avramov, I. Tchalakov, T. Stefanova, M. Chouchkova, Sofia*

**GAM24** **Purification and partial characterization of cold-active enzyme superoxide dismutase**
*R.Abrashev, L. Stefanova, B.Spassova, V. Dishliiska, S. Pashova, M. Angelova, Sofia*

**GAM25** **Preparation, performance and application of Aspergil lus niger B 03 endo-xylanase immobilized on the smart polymers eudragit S-100 and L-100**
*G. Delcheva, B. Zhekova, G. Dobrev, I. Pishtiyski, Plovdiv*

**GAM26** **Biochemical characterization of recombinant strains of Lactobacillus BA68, Lactobacillus RB44, Lactobacillus RD13**
*I. Dobrev, Z. Denkova, I. Murgov, Plovdiv*

**GAM27** **Antioxidant activities and bioactive compounds in extracts of Salvia tomentosa mill. plant and cell suspension cultures**
*A. Marchev, V. Georgiev, M. Nikolova, I. Ivanov, A. Pavlov, Plovdiv, Sofia*

**GAM28** **Optimization of the composition of the medium for obtaining lactic acid with Lactobacillus helveticus ssp. rhamnosus NBIMCC 507**
*B. Goranov, M. Angelov, I. Dobrev, G. Kostov, Z. Denkova, Plovdiv*

**GAM29** **Anaerobic digestion of cellulose containing wastes in mesophillic conditions**
*V.Hubenov, V. Dencheva, D. Denchev, I. Simeonov, Sofia*

**GAM30** **Bioreactor productions of galanthamine by Leucojum aestivum L. shoot culture. a comparative study**
*I. Ivanov, V. Georgiev, M. Georgiev, M. Ilieva, S. Berkov, A. Pavlov, Plovdiv, Sofia*

**GAM31** **Decolorization of different classes of dyes by immobilized mycelia of Trametes versicolor**
*A. Krastanov, R. Koleva, I. Stoilova, Plovdiv*

**GAM32** **Bulgarian reference reagent for BCG vaccine: viabilty evaluated by the bioluminescent ATP-assay and viable count method**
*E.L. Pavlova,. T.R Stefanova, A.E. Mihaylov, C.M. Shouchkova, V.M. Savov, V.I. Iordanova,. R.M Dimitrova, Sofia*

**GAM33** **Anaerobic digestion of cattle dung in bioprocess system with fixed film**
*E. Chorukova, V. Mamatarkova, I. Simenonov, L. Nikolov, Sofia*

**GAM34** **Comparative assessment of the antioxidant capacity of LACTOCOCCUS Lactis ssp. lactis and Propionibacterium freudenreichii ssp.shermanii**
*V. Yanakieva, Z. Denkova, I. Murgov, Plovdiv*

**GAM35** **Obtaining lactic acid with immobilized lactic acid bacteria**
*M. Angelov, Z. Denkova, G. Kostov, B. Goranov, I. Dobrev, Plovdiv*

**GAM36** **Comparative study of obtaining of lactic acid with free and immobilized cells of Lactobacillus casei ssp. rhamnosus NBIMCC 1013**
*M. Angelov, P. Dimitrova, B. Goranov, G. Kostov, Z. Denkova, Plovdiv*

**GAM37** **Selected strains with intestinal origin and from bulgarian yoghurt inhibit TGF-beta and interleukin-8 secretion from epitheal cells, and induce secretion of interleukin-10 from macrophages**
*Z. Dimitrov, Sofia*

**GAM38** **Development of strain discriminative AFLP for lactobacilli with intestinal origin**
*Z. Dimitrov, Sofia*

**GAM39** **Characterization of activity and expression of virulence factors of eneterococci isolated from traditional bulgarian cheeses**
*N. Kirilov, I. Iliev, I. Ivanova, Sofia, Plovdiv*

**GAM40** **Assimilation of cholesterol by lactic acid bacteria**
*J. Atanasova, D. Yordanova, Z. Urshev, I. Ivanova, Z. Nikolov, Sofia*

**GAM41** **Phytopathopgenic bacteria of genus Burkholderia in Bulgaria**
*Y. Kizheva, P. Hristova, N. Bogatzevska, S. Tishkov, A. Doycheva, V. Chipeva, P. Moncheva, Sofia, Kostinbrod*

**GAM42** **Cu (II) stress response of growing Aspergillus niger cells**
*D. Todorova, K. Tsekova, Sofia*

**GAM43** **Thermophysical and IR spectral characteristics of an exopolysaccharide synthesized by the psychrophilic yeast isolate 102**
*I. Panchev, K. Pavlova, S. Rusinova-Videva, M. Kuncheva, Plovdiv*

**GAM44** **PCR detection and 16S RDNA sequence analysis of different Acidithiobacillus ferrooxidans isolates**
*V. Groudeva,. R. Pironkova, M. Iliev, Sofia*

**GAM45** **Biodiversity of iron bacteria in natural habitats in vitosha mountain**
*V. Groudeva, R. Pironkova, A. Doycheva, M. Iliev, Sofia*

**GAM46** **Microbial colonization and identification of bacterial strains isolated from thracian vaults near Kasanlak, Bulgaria**
*V. Groudeva, R. Pironkova, Krumova, A. Doycheva, M. Iliev., Sofia*

**GAM47** **Long - term studies on the microflora and microbial processes in a dump consisting of copper sulphide ore**
*V. Groudeva, M. Iliev, A. Doycheva, S. Groudev, Sofia*

**GAM48** **Bioremediation of waters polluted with heavy metals, arsenic and crude oil by means of a passive system**
*V. Groudeva, M. Iliev, A. Doycheva, S. Groudev, Sofia*

**GAM49** **Comparative studies on some probiotic properties of three strains of Bifidobacterium longum from human origin**
*K. Pashova-Baltova, R. Naidenova, Z. Dimitrov, Z. Urshev, Sofia*

**GAM50** **Degradation of mixed aromatic pollutants by trametes versicolor strain**
*Z. Alexieva, H. Yemendzhiev, B. Atanasov, A. Terziyska, A. Krastanov, Sofia, Plovdiv*

**GAM51** **Sequence analysis of gene coding for phenol hydroxylase in Aspergillus awamori strain**
*H. Yemendzhiev, J. Manasiev, Z. Alexieva, Sofia*

**GAM52** **Decolorization of textile dye remazol brilliant blue r by mycelial culture of white-rot fungi Trametes versicolor 1**
*H. Yemendzhiev, A. Krastanov, N. Peneva, N. Shivarova, Z. Alexieva, Sofia, Plovdiv*

**GAM53** **Reversibility and stability of superoxide dismutase Humicola lutea 103**
*P. Dolashka-Angelova, G. Subba Rao, V. Moshtanska, L.Velkova, B. Atanasov, A. Dolashki, M. Angelova, W.Voelter, Sofia, New Delhi, Tübingen,*

**Great Hall: Virology**

**V22** **Study of diagnostic value of RNA-based HPV test NucliSENS EasyQtm HPV**
*M. Boyanova, D. Velcheva, D. Tsoncheva, P. Kostova, A. Doganov, V. Zlatkov, Sofia*

**V23** **Fluorescence antibody labeling applied to cells infected with viruses and bacteria**
*V Levakova, A.S.Galabov, Sofia*

**V24** **Antioxidant prevention as a factor reducing stress ulcers in acute radiation syndrome**
*I. Kindekov, I., M. Mileva , A. Galabov , V.Vassilieva, M.Alyakov, Sofia*

**V25** **The difficult strugle against measles**
*Ch. Odisseev, Z. Mihneva, Sofia*

## Wednesday, October 13

**Great Hall: Medical Microbiology**

**16:00 – 17:30**

**MM11** **Microbiological control of nose, mouth and throat swabs as a measure of obligatory health supervision**
*S. Stoilova, M. Stoilov, Bitola*

**MM12** **Controlling inanimate environment as a measure for enforcing the health surveillance law**
*L. Petrulovska, B. Petrulovska, Skopje*

**MM13** **Effective real time PCR SYBR green for early and rapid detection of Coxiella burnetii**
*M. Kunchev , D. Alexandrova , K. Mladenov, K. Mekouchinov, Sofia*

**MM14** **Modern approaches to a rapid detection of Mycobacterium tuberculosis strains in Bulgaria**
*V. Valcheva, Sofia*

**MM15** **Some aspects in virulence of Echerichia coli, associated with urinary tract infections**
*M. Marhova, R. Ivanova, S. Kostadinova, Plovdiv*

**MM16** **Fungicidal activity of hybrid materials based on polyvinylpyrrolidone with embedded silver nanoparticles**
*D. Pencheva, T. Kantardjiev, R. Bryaskova, Sofia*

**MM17** **Comparisin the fungicidal properties of silver nanoparticles syntethesized via chemical and thermal reduction of silver nitrate in the presence of different polymers as stabilizing agents**
*D. Pencheva, T. Kantardjiev, R. Bryaskova, Sofia*

**MM18** **Quality testing of commercially prepared (Bul Bio - NCIPD) microbiological culture media, according to the requirements of ISO/TS 11133**
*D. Pencheva, M. Rumenkina, D. Chankova, M. Nikolova, Sofia*

**MM19** **Туларемията в България**
*В. Късовски, Н. Готев, К. Младенов, Т. Кантарджиев, И. Иванов, Т. Димитрова, В. Дойчева , Sofia*

**MM20** **Сто години от изолирането на причинителя на туларемията**
*А. Гълъбов, Т. Кантарджиев, Н. Готев, В. Дойчева, Sofia*

**Veterinary Microbiology**

**11:30 – 12:00**

**VM5** **Comparison of the methods for the tuberculosis proof by the farm animals**
*M. Bonovska, T. Savova, Y. Petkov, M. Dragoycheva, Sofia*

**VM6** **Enhanced resistance against Salmonella typhimurium infection in chicken, treated with a bacterial product (BP)**
*H. Neychev, L. Doumanova, Y. Neycheva, Z. Stefanova, P. Stoyanova, A. Angelov, Sofia, Bulgaria*

**Plant and Soil Microbiology**

**09:30-11.30**

**PSM4** **Phytopathogenic Serratia rubidea isolated from tulip.**
*M. Stoyanova and N. Bogatzevska, Kostinbrod*

**PSM5** **New approaches for agrobacterium-mediated genetic transformation of plant cells**
*A. Marchev, V. Georgiev, I. Ivanov and A. Pavlov, Plovdiv*

**PSM6** **Research on the reaction of plum cultivars to Polystigma rubrum /(Persoon) De Candole/**
*P. Iliev and A. Stoev, Dryanovo, Kostinbrod*

**PSM7** **Spring oat cultivars and lines as hosts of viruses in natural condition (preliminary screening)**
*N. Bakardjieva, A. Stoev and T. Savova, Kostinbrod, Karnobat*

**PSM8** **A new method for distinguishing Burkholderia gladioli pathovars from plant samples.**
*M. Stoyanova, P. Hristova, N. Petrov and Bogatzevska, Kostinbrod, Sofia*

**PSM9** **In vitro and in vivo production of dsRNA for RNA interference targeting HC-Pro gene region of PVY.**
*N. Petrov and M. Stoyanova, Kostinbrod*

**PSM10** **In vitro antibacterial and antifungal activity of different species and strains microalgae.**
*H. Najdenski, L. Gigova, I. Tsvetkova, M. Ninova and V. Kusssovski, Sofia*

**PSM11** **Morphological and biological characteristics of entomopatogenical viruses (baculovirus) isolated and studied in Bulgaria**
*A. Atanassov, Kostinbrod*

**11:30-12:00** General discussion

**Infectious Immunology**

**10:30 – 11:30**

**II 1** **Ibogaine confers protection in murine Candida albicans sepsis**
*Petya Dimitrova, Nina Ivanovska*

**II 2** **Antigenic properties of yersinia outer membrane proteins**
*Elica Golkocheva-Markova, Rumen Stoilov, Hristo Najdenski*

**II 3** **Application of tyrphostin ag-490 in lps-induced shock**
*Valeriya Gyurkovska*

**II 4** **7-hydroxycoumarin influences the immune response of chickens infected with salmonella typhimurium**
*H. Neychev, Y. Neycheva, Z. Stefanova, P. Stoyanova, A. Angelov.*

# ABSRTACTS

# GENERAL AND APPLIED MICROBIOLOGY

## GAM1

## COMPATIBLE SOLUTES IN THERMOPHILES AND HYPERTHERMOPHILES FROM THE ESOTERIC TO THE APPLIED

Milton S. da Costa and Nuno Empadinhas
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Many organisms that live at very high temperatures have been isolated from shallow marine and abyssal thermal environments, where the geothermal water may reach the salinity of the surrounding seawater. These organisms, like all other microorganisms must adjust, within intrinsic limits, to alterations in the water activity of the environments. The majority of microorganisms adjust osmotically by the selective accumulation of small molecular weight organic compounds. Thermophiles and hyperthermophiles accumulate a few compatible solutes that are also common in mesophilic bacteria and archaea, namely trehalose and glutamate and even the very rare glucosylglycerate (GG). However, the majority of the compatible solutes encountered in hyper/thermophiles are unique to these organisms. These compatible solutes include mannosylglycerate, di-myo-inositol-phosphate and the very rare compatible solutes di-glycerol-phosphate, di-mannosyl-di-myo-inositol-phosphate and mannosylglyceramide.

In recent years we have studied the synthesis of mannosylglycerate (MG) and trehalose in *Thermus thermophilus* (Phylum *Deinococcus*/*Thermus*), *Rubrobacter xylanophilus* (Phylum *Actinobacteria*) and *Persephonella marina* (Order *Aquificales*). The species of the genus *Thermus* have optimum growth temperatures that range between 70 and 75ºC and, with the exception of *Thermus thermophilus*, a maximum growth temperature below 80ºC. The species *Thermus thermophilus* have a maximum growth temperature of about 82 to 83ºC. This species is also capable of growing in media containing 3 to 5% NaCl. The strains of *T. thermophilus* accumulate primarily trehalose and lower levels of mannosylglycerate (MG) during osmotic adjustment. Recombinant mutants lacking the genes for the synthesis of trehalose, MG or both, result in a profound effect on the ability of organisms to grow in media

containing NaCl. The synthesis of MG by *T. thermophilus* proceeds via a two step pathway catalyzed by mannosyl-phosphoglycerate synthase (MpgS) and mannosyl-phosphoglycerate phosphatase (MpgP) from GDP-mannose and 3-phosphoglycerate. These enzymes are very similar to those found in other hyper/thermophilic organisms, however the MpgS and the MpgP from *R. xylanophilus* have little or no identity to the previous enzymes. The homologous enzymes of *R. xylanophilus* lead, depending of the substrate, to the synthesis of MG or GG. The thermophilic bacterium *P. marina*, on the other hand, is the only known thermophile to accumulate GG in response to salt stress and possesses genes that are also very different from the ones mentioned above. Moreover, this organism possesses two pathways for the synthesis of GG. The physiological relevance of MG and GG accumulation in these thermophilic bacteria and the evolution of MG and GG biosynthesis in prokaryotes are discussed. These studies led us to encounter a gene in the species of Mycobacterium that leads to the synthesis of GG that is bound to a polysaccharide and does not serve as a compatible solute.

## GAM2

## GRAM NEGATIVE LIPOPOLYSACCHARIDE AND PEPTIDOGLYCAN AS ELICITORS OF INNATE IMMUNITY SYSTEM IN MAMMALS AND PLANTS

Antonio Molinaro
Dipartimento di Chimica Organica e Biochimica, Universita di Napoli Federico II, Napoli, Itali

Innate immunity is the first line of defence against invading microorganisms in vertebrates and the only line of defence in invertebrates and plants and therefore plays a crucial role in the early recognition and subsequent triggering of a pro-inflammatory response to invading pathogens.

This mechanism relies on recognition of evolutionarily conserved structures on pathogens, termed microbe-associated molecular patterns (MAMPs), through a limited number of germ line-encoded pattern recognition receptors. MAMPs are characterized by being invariant among entire classes of pathogens, essential for the survival of the pathogen, and distinguishable from "self".

Gram negative lipopolysaccharide and peptidoglycan are two very important cell wall glyco-conjugates and act as MAMPs in eukaryotic/bacteria

interactions. Besides their general architectural principle, a number of subtle chemical variations are at the basis of the dynamic host-guest recognition that in case of pathogens is followed by the innate response and in case of symbiosis is followed by its suppression. Therefore, the structural study of such glyco-conjugates involved as virulence factors in animal or plant infections is a pivotal pre-requisite for the comprehension at molecular level of the innate immunity mechanisms.

In this communication I will show some examples of isolation, structure determination and elicitation and/or suppression of plant and animal innate immunity by peptidoglycan and lipopolysaccharides from pathogen and symbiotic Gram negative bacteria.

## GAM3

## APPLICATION OF PROBIOTIC BACTERIA IN FUNCTIONAL FOODS

Z. Denkova, I. Murgov
University of Food Technologies, 26 Maritza Blvd, 4002 Plovdiv, Bulgaria

The inclusion of probiotic lactic acid bacteria and bifidobacteria in foods allows increasing of their biological value and becoming functional foods. Strains of lactobacilli and bifidobacteria with probiotic properties, which are included as components of starter cultures for fermented milk and soy products, as well as starter cultures for the meat industry have been selected.

The obtained probiotic starter cultures have been embedded in the production of lactic acid products, soy products and meat raw-dried and brew-smoldered foods.

The technology for Bulgarian yoghurt with a high concentration of *Lactobacillus delbrueckii subsp. bulgaricus*, bifid yogurt and lactic acid drinks, soy yoghurts (natural, acidophilic, bifid) has been developed and implemented in practice. Increased durability of fermented products' storage (over 30 days) at 4 ± 2 ° C and maintaining the content of active cells and the acidity of the products have been achieved.

After consumption of fermented food the necessary amount of beneficial probiotic microflora to maintain the balance in the digestive tract is delivered to the body.

The inclusion of lactobacilli with probiotic properties in the composition of starter cultures for raw-dried and brew-smoldered meat products provides

purposeful fermentation process, safety of the ready meat products and an improved sensory profile. A high content of viable cells of probiotic bacteria in the ready products has been observed. Along with meat foods they fall within the gastrointestinal tract and through their vital activity they improve the balance of microflora in the stomach and the intestines.

## GAM4

# METABOLIC ALTERATIONS DURING GROWTH OF *LACTOBACILLUS* STRAINS IN THE PRESENCE OF OLIGOSACCHARIDES AND WHEY PROTEINS

T. Mandadzhieva[1], Ts. Ignatova-Ivanova[2], I. Iliev [3], I. Ivanova [1]
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*2 Department of Functional Biology, Schoumen University, Schoumen, Bulgaria*
*3 Department of Biochemistry and Microbiology, Plovdiv University, Plovdiv, Bulgaria*
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Prebiotics are short-chain carbohydrates (SCCs) that are non-digestible by human enzymes and that have been digested by Lactic acid bacteria and Bifidobacteria. Both the structure of the carbohydrate and the bacterial species present in the eco-system are probably important factor in controlling the fermentation of SCCs. The mechanisms by which prebiotic oligosaccharides are selectively metabolized by beneficial members of the gut microbiota are not adequately understood at the present time.

The aim of the present work is to look at whether a combination of oligosaccharides and peptides released after cultivation in whey media would produce a synergistic effect in antimicrobial activities of *Lactobacillus* strains.

More than 40 strains affiliated to the genera *Lactobacillus*, were tested for their ability to grow on different oligosaccharides. The unusual sugars influenced in a specific way the production of antimicrobials, active against different pathogens. The obtained data suggest that the studied lactic acid strains are able also to change the fermentation patterns, depending on the available substrates and the metabolitic pathways of the strains. The cultivation of these strains in whey proteins contributed to higher antimicrobial activity too. It could be speculated that the combination of an oligosaccharide and an antimi-

crobial substance would produce a synergistic effect in promoting the growth of positive bacteria in the gut. The exploitation of the diversity of lactic acid bacteria from traditional fermentations remain an important resource for identification of novel metabolic traits of LAB for use in the production of value added food ingredients.

**Acknowledgements:** We would like to express our gratitude for the support of this work by research grant of NSF Bulgaria RNF02-02-2009 and BG051PO001-3.3.04/32 HR Project.

## GAM5

## НАЧИНИ ЗА ТРАНСПОРТ НА МИКРОБИОЛОГИЧНИ ПРОБИ ЗА ИЗСЛЕДВАНЕ И АНАЛИЗ

*World Courier Bulgaria Ltd., Sofia*

## GAM6

## МАСС ПЕКТРОМЕТЪРЪТ MALDI BIOTYPER (BRUKER DALTONICS, GERMANY) – МИКРОБИАЛНАТА ИДЕНТИФИКАЦИЯ НА XXI ВЕК

*N. Bajnov, Medical Technics Engineering Ltd, Sofia*

## GAM7

## DEVELOPMENTS AND ACHIEVEMENTS IN MICROBIAL PRODUCTION OF AMINO ACIDS AT THE INSTITUTE OF MICROBIOLOGY – BAS

Alexander Ratkov
The Stephan Angeloff Institute of Microbiology - Bulgarian Academy of Science

The amino acids, as the building blocks of life, have long played an impor-

tant role in both human and animal nutrition and health maintenance. In the 1950s *Corynebacterium glutamicum* was found to be a very efficient producer of L-glutamic acid. Since this time biotechnological production processes have been used for industrial production of amino acids. At present these processes are among the most important in terms of tonnage and economical value. Market development has been particularly dynamic for the flavor-enhancer glutamic acid and the animal feed amino acids L-lysine, L-threonine, and L-tryptophan. The growing market for amino acid led to significant improvements in bioprocess and downstream technology as well as in molecular biology. During the last decade big efforts were made to increase the productivity and to decrease the production cost.

This presentation gives an overview of the world market for amino acids. Attempt and achievements in investigations and developments of the technologies for amino acids production at the Institute of Microbiology, Bulgarian Academy of Sciences are summarized. Improvements in selection of different microbial strain-producers for different amino acids, as well as developments and achievements in bioprocess technology, i.e. development of lab scale, semi-industrial scale and production scale technologies for production of some amino acids are summarized too. Specificity and efficiency of applying of fed-batch and repeated fed batch (fed-batch with droppings) cultivation of the producers is shortly discussed.

## GAM8

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

I. Simeonov, D. Denchev
The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Science

The paper deals with anaerobic digestion of different organic wastes (single and mixtures) in different types of bioreactors. The state of the art of this anaerobic biotechnology is presented in different countries and the latest results of the multidisciplinary team of the Institute of Microbiology"St. Angeloff" – BAS, as well. Some new comparative studies with a classical CSTR bioreactor with suspended culture for biogas production and a new submerged packed bed biofilm reactor are presented and some new results concerning the

biogas production in meso- and thermophillic conditions, as well. One of the latest problems in this field - anaerobic digestion of cellulose contained wastes is discussed and some new results are presented. Finally some new perspectives in this area are presented and discussed.

**Acknowledgments:** This work is supported by the NSF, Bulgaria, Contract DO 02-190/08.

## GAM9

## BIOTECHNOLOGICAL PRODUCTION OF GALANTHAMINE

Ivan Ivanov[1], Vasil Georgiev[1], Mladenka Ilieva[1], Strahil Berkov[2], **Atanas Pavlov[1]**
[1]The Stephan Angeloff Institute of Microbiology - Bulgarian Academy of Science, Laboratory of Applied biotechnologies; [2]AgroBioInstitute

From time immemorial, humans have been highly dependent on plants as sources of proteins, carbohydrates and fats. Furthermore, the volume and range of phytochemicals used by modern society (inter alia as drugs, nutrients, cosmetic additives and biopesticides) are continuously expanding. These high demands are driving efforts to develop new ways to produce plant-derived metabolites. Plant *in vitro* techniques, in which plant cells, tissue and organs are cultivated in sterile conditions totally offer such alternatives for producing important metabolites.

Galanthamine is a long acting acetylcholinesterase inhibitor marketed for the treatment of Alzheimer's disease. Although chemical synthesis of galanthamine has been successfully performed, the main commercial sources for the production of the galanthamine-based medicines are Amaryllidaceae plants. Nowadays, prescription regime of the utilization of more of these plant species has been imposed and, from these perspectives, galanthamine production from *in vitro* cultures is considered as an attractive alternative.

The lecture will attend to alternative biotechnological approach for galanthamine production with *Leucojum aestivum* L. shoot culture.

**Acknowledgment**: This research was supported by National Science fund of Bulgarian under contract DO/02/105-2009

**GAM10**

# DETERMINATION OF CAPSULAR POLYSACCHARIDES IN TWO CLINICAL ISOLATES OF *ACINETOBACTER BAUMANNII*, SMAL AND MG1

Eleonora Fregolino, Otto Holst, Rosa Lanzetta, Michelangelo Parrilli, Cristina De Castro
Dipartimento di Chimica Organica e Biochimica, Universita di Napoli Federico II, Napoli, Itali

Bacteria of genus Acinetobacter (family of *Moraxellaceae*, subclass of *Proteobacteria*) are ubiquitous and different species have been isolated from clinical specimens, in particular *Acinetobacter baumannii*. The mechanism used by *Acinetobacter* to express its pathogenicity is not completely elucidated, but it is known that a crucial role is played by capsular polysaccharides (CPSs) and LPSs.

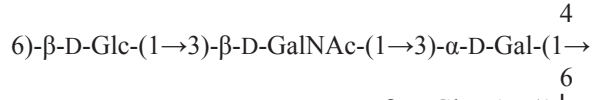
Ongoing studies are attempting to realize a serotyping scheme of this bacterium analogue to that in use for *E. coli*; analyzing both the capsular polysaccharides (CPS) and the lipopolysaccharides (LPS): the main difficulty of this work resides in the speciation of the material isolated, namely if the polysaccharide isolated belongs to the O-antigen of the LPS or to the CPS.

In the present work, the characterization of the CPS from two clinical isolates of *A. Baumannii* (strains SMAL and MG1), was established with immunological methods after extraction and purification of the polysaccharides.

The CPSs were analyzed via NMR spectroscopy and GC-MS and resulted in the following structures, information at the basis for the elaboration of a serotyping scheme:

MG1: 4)-α-D-GlcNAc-(1→4)-α-L-GalNAcA-(1→3)-β-D-QuinNAc4NR-(1→
R= acetyl or 3-HBA

SMAL:



## GAM11

# BIOFILMS OF ESCHERICHIA COLI: EFFECTS OF SECRETED METABOLITES OF OTHER BACTERIA ON GROWTH AND DEVELOPMENT

S. Stoitsova[1], A. Vacheva[1], S. Danova[1], R. Georgieva[1], R. Ivanova[1], S. Kostadinova[2], M. Marhova[2], K. Vasileva[1], T. Velinov[3], M. Bivolarska[3], Y. Baldjiska[3]
[1]The Stephan Angeloff Institute of Microbiology, BAS;
[2]Plovdiv University 'P. Hilendarski';
[3]Sofia University 'St Kliment Ohridski'

In nature, microorganisms most often exist as biofilms attached on surfaces. These may be formed by single species, or, in most cases, be more complex consortia of different bacterial species or/and eukaryotic organisms. This suggests complicated interstrain and interspecies interaction via both cell-to-cell closer contacts and, more remotely, by secretory metabolites. The report summarizes results on the effects of sterile stationary-phase supernatants of other bacteria on biofilm growth and development by laboratory strains of *E. coli* and clinically isolated UPEC. Dependent on strain combinations, effects on *E. coli* biofilm may vary from stimulation, lack of effect, to suppression. Thus, *E. coli* K-12 strains W1655, 420, and 406, produce significantly greater biofilm biomass when grown in M63 medium, supplemented with spent media from late stationary-phase cultures of *E. coli* K-12, *E. coli* O157, or *Y. enterocolitica*. What is more, strains that do not produce detectable amounts of biofilm in pure M63 medium, like *E. coli* 421 and 446, are stimulated for sessile growth by the spent culture media of commensal and pathogenic representatives of *Enterobacteriaceae*. The effects concern both early attachment and biofilm morphogenesis, however later on may speed up initiation of biofilm detachment. Spent culture media of lactobacilli with pre-estimated probiotic potential have also been applied as supplements during biofilm growth, with a variety of outcomes. Among these, the products from *L. salivarius* LB1, *L. fermentum* LB5 and *L. plantarum* LB10 inhibited biofilm in all tested laboratory and uropathogenic *E. coli* strains. Taken together, the results show variability of responses to similar treatments within individual *E. coli* strains. This may be due to strain-to-strain differences in the strategies for substrate occupation by *E. coli*, and stresses on difficulties in identifying universal biofilm inhibitors applicable in practice.

**Acknowledgements**: This study was funded by the National Science Fund

of Bulgaria, Contract VU-L-321/07. The support of A. Vacheva and R. Georgieva by BG051PO001-3.3.04/32 HR Project should also be acknowledged.

## GAM12

# *IN VITRO* SELECTION OF LACTOBACILLI WITH PROBIOTIC POTENTIAL FOR DEVELOPMENT OF NEW FUNCTIONAL FOODS AND THERAPEUTIC FORMULAS

Svetla Danova[1*], Ralitsa Georgieva[1], Rositsa Tropcheva[1], Jordan Manassiev[1], Jordanka Dermendjieva, Nina Ivanovska[2]
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Human desire for long healthy life is creating needs for improvement of food practices of the modern societies, as well as for new approaches in prophylactic and therapy of different disorders. Thus, a growing interest of scientists on the potential of beneficial lactic acid bacteria (LAB) and especially lactobacilli have been observed. With this aim more than 10 years research in the Institute of Microbiology allows isolation and characterization of lactobacilli from different ecological niches. A collection with 200 LAB strains was created and i*n vitro* selection criteria (FDA/WHO) are applied to select the strains with potential of good bacteria, coffering direct benefits for their hosts. The probiotic potential of several *Lactobacillus* strains with human and dairy origin was estimated. New isolates belonging to the species *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus salivarius* and *Lactobacillus brevis* were evaluated as prospective bio-protective cultures with broad spectrum of anti-microbial activity. In addition, good transit tolerance in simulated GIT conditions, adhesion capacity and protective bio-film formation was proved in different model systems. Molecular mechanisms of their biological and protective/immunomodulation activity were studied. Some of selected LAB cultures possess technologically relevant properties.

Obtained *in vitro* results implied that tested lactobacilli are appropriate for further development of multifunctional probiotic formulas.

**Acknowledgements**: This work was supported by grant DOO2-187/2008 of National Science Fund, and BG051PO001-3.3.04/32 HR Project, Bulgaria

## GAM13

# BIOTECHNOLOGICAL PRODUCTION OF COLD-ACTIVE SUPEROXIDE DISMUTASE BY ANTARCTIC FUNGUS *ASPERGILUS GLAUCUS* 363

V. Dishliyska[1], S. Pashova[1], L. Stefanova[2], K. Krumova[1], Z. Alexieva[2], H. Jemendzhiev[2],
B. Spassova[1], S. Vassilev[3], M. Angelova[1]
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The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

Novel cold-active or cold adapted (CA) enzymes may be produced by organisms existing in permanently cold habitats. Antarctic microorganisms, including filamentous fungi, live at the absolute edge of life under the most extreme conditions known on Earth. They could be a source of such CA enzymes, which offer economic benefits through energy savings and significant advantages at the level of their specific activity, lower stability and unusual specificity.The superoxide dismutases (SODs) are a group of metalloenzymes which function appears to be protection of cells from the toxic effects of the endogenously generated superoxide radicals. A CA SOD with optimum activity near human body physiological temperature would have great advantages in medicine, in cryopreservation processes and cryosurgery, different cosmetic formulations and skin care products.

Filamentous fungi isolated from soil samples taken from Livingston Island, Antarctica were screened for CA SOD production. The best producer, isolate 363, was identified by using 16S rDNA sequencing and found to belong to a species *Aspergillus glaucus*. The optimum fermentation conditions for enzyme production in 3 L bioreactors were examined. Results showed that the optimum temperature was at 25ºC and incubation period required for maximum production was 24 h with stirrer speed of 400 rpm air flow, 1 v.v.m. CA SOD synthetized by *A. glaucus* 363 was significantly more thermo sensitive than the mesophilic SOD and remained 100% activity at 10°C.

**Acknowledgements**: This work was supported by the European Social Fund, Operational Programme "Human Resources Development" (grant BG051PO001-3.3.04/32) and National Scientific Fund of the Ministry of Education and Science, Bulgaria (grant DO02-172/08).

**GAM14**

# BIOCHEMICAL AND GENETIC STUDIES ON THE PRODUCTION OF NEURAMINIDASE BY ENVIRONMENTAL ISOLATES OF *VIBRIO CHOLERAE NON-O1* FROM BULGARIA

R. Eneva[1], S. Engibarov [1], T. Strateva[2], R. Abrashev [1], I. Abrashev[1]*
[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria;
[2]Department of Medical Microbiology, Medical University of Sofia
*Corresponding author: Ignat Abrashev, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia 1113, Acad. G. Bonchev str. Bl 26, Bulgaria, abrashev@microbio.bas.bg

Neuraminidase is an important instrument in the structural analysis of glycoproteins, glycolipids and oligosaccharides. It is a key factor in the infectious process of many viruses and pathogenic bacteria. Neuraminidase, secreted by the etiological agent of cholera - *Vibrio cholerae* O1 is well studied, in contrast with the enzyme, produced by non-O1/non-O139 *V. cholerae.* In this study environmental non-O1/non-O139 *V. cholerae* isolates from Bulgaria are screened for production of neuraminidase. Enzyme activity and presence of neuraminidase gene *nanH* is detected in 5 strains. One of them – *V. cholerae* non-O1/13 was used to investigate the enzyme production in several media and at different aeration conditions. Its intracellular neuraminidase activity was also assayed.

**Key words**: *neuraminidase, Vibrio cholerae non-O1, nanH gene*

**GAM15**

# COLD ADAPTATION AND OXIDATIVE STRESS RESPONSE OF TWO FILAMENTOUS FUNGI, ISOLATED FROM LIVINGSTON ISLAND, ANTARCTICA

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There is a hypothesis that low temperature induced oxidative stress, which has greater toxicity potentials on all cell biomolecules. We examine the relationship between short-term exposure to cold shock and the level of oxidative stress biomarkers and antioxidant enzyme defense. Two fungal strains, isolated from Livingston Island, Antarctica (*Penicillium* sp. 161, psychrotolerant and *Aspergillus glaucus* 363, mesophilic) were treated by cold temperatures for 6 h and their cell response was determined. Downshift from an optimal temperature to 4 or 10°C led to events typical of oxidative stress: significant reduction of biomass production; increase in the levels of oxidative damaged proteins and accumulation of storage carbohydrates (glycogen and trehalose) in comparison to growth at optimal temperature. Cell response against cold stress includes also increase in the activities of SOD and CAT, which are key enzymes for directly scavenging reactive oxygen species. This response is more species-dependent than dependent on the degree of cold shock. Antarctic psychrotolerant strain *Penicillium* sp. 161 that is adapted to life in extremely cold conditions demonstrated enhanced tolerance to temperature downshift in comparison with Antarctic mesophilic strain (*Aspergillus glaucus* 363).

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## GAM16

# MOLECULAR CHARACTERIZATION OF BACTERIAL CULTURES ISOLATED FROM XENOBIOTIC POLLUTED ENVIRONMENT AND ITS POSSIBLE APPLICATION IN BIOREMEDIATION TECHNOLOGIES

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Microbial cultures isolated from harshly polluted with heavy metals and hydrocarbons environments were investigated using 16S rDNA approach. Isolates KCM R5 and KCM RG5 were cultured from the vicinity of Pb-Zn smelter, Plovdiv, Bulgaria. The strain BT 271 was isolated from phenol-polluted soil in the region of the petrochemical plant near Bourgas and AP 9 – from activated sludge of Sofia Waste water treatment plant. Our analysis of the 16S rRNA gene of the isolates showed that the sequences of KCM $R_5$ and AP 9 were affiliated with over 99 % identity to the 16S rRNA gene of *Pseudomonas* sp. while KCM RG5 and BT 271 sequences were closely related to 16S rRNA gene of *Bacillus* sp. All characterized strains were subjected on high selective pressure in order to increase their xenobiotic degrading potential.

All strains demonstrated capability for utilization of aryl-containing xenobiotics including phenol, *o*-, *m*-, *p*-nitrophenols, 2,4-dinitrophenol, 2,5-dinitrophenol, pentachlorophenol, and 2,4-dichlorophenoxiacetic acid. Recently KCM R5, KCM RG5, BT 271 and AP 9 were entrapped in polymer matrices as poly(ethylene)oxide called PEO-criogels. PEO cryogels were prepared by P. Petrov and Ch. Tsvetanov (Institute of Polymers, BAS, Sofia) following an original method. The immobilized biosystems were included in model process in laboratory biofilter and were studied for phenol degradation in semicontinious processes. Our results showed that so constructed biotechnological systems are sustainable and can degrade phenol in increasing concentration in flow from 300 to 1000 mg $L^{-1}$ with high effectiveness (90 – 100%). The results confirmed that new material, selected bacterial cultures, and specialized algorithm of the waste water treatment process (special biofilter and parameters of the process) are a prospective innovation for application in the bioremediation detoxication technologies of industrially polluted waters.

**GAM17**

# ANTIMICROBIAL POTENTIAL OF SELECTED THIOSULFONATES–BASED BIOCIDES AND BIOSURFACTANTS AGAINST BACTERIA AND FUNGI

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Disease-causing microorganisms resistant to antibiotic and antimicrobial drug therapy are an increasing public problem. Some organisms are resistant to all approved antibiotics and can be treated with experimental and potentially toxic drugs only. The need of new antimicrobial agents to overcome microbial antibiotic tolerance or resistance leads to investigations towards novel strategies to fight off microbial infections

Antimicrobial potential of newly synthesized alkyl esters of thiosulfonic acid-ethyltiosulfonate (ETS) and methyltiosulfonate (MTS) in absence and in presence of rhamnolipid- biosurfactant was investigated against model bacterial and fungal strains: *Bacillus subtilis, Pseudomonas aeruginosa, Alcaligenes faecalis* and *Rhizopus nigricans*. The results demonstrated that the bactericidal and fungicidal effects of both inhibitors were well expressed. Methyltiosulfonate had a stronger antimicrobial effect. The presence of rhamnolipid- biosurfactant enhanced the bactericidal, sporocidal and fungicidal effect of the inhibitors.

In practical terms MTS and ETS could be used in industry to enhance the effectiveness of the production process avoiding additional contamination of the environment as well; in agriculture to achieve higher yields, minimizing the presence of harmful phytopathogens and in biomedicine for new antimicrobial drug therapy. The presence of rhamnolipid-biosurfactant in new antimicrobial preparations on the base of MTS and ETS makes them more effective.

## GAM18

# BIOFILM-RELATED PHENOTYPES IN *ESCHERICHIA COLI* K-12: EFFECTS OF LATE STATIONARY PHASE SUPERNATANTS

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In most natural environments bacteria associate with a surface and form structures known as biofilms. In the *Escherichia coli* strains examined so far the expression of different phenotypes has been reported in association with biofilm formation. This study aims at the examination of the effect of sterile stationary-phase supernatants containing secretory metabolite products by three species of *Enterobacteriaceae* (SMP-E) on the expression of biofilm-related phenotypes of *E. coli* K-12 strains. Materials and methods: Eight strains *E. coli* K-12 were tested (three – good biofilm producers, two – shown to produce biofilm only in the presence of SMP-E, and three that produced no biofilm under the tested experimental scheme). Motility was examined on SMP-E – supplemented with 0.3 % motility agar. Cellulose and curli synthesis was checked on congo red agar. The expression of type I fimbriae was demonstrated by co-aggregation test between *E. coli* and *Saccharomyces serevisiae*. The production of the exopolysaccharides colanic acid (CA) and poly N-acetyl-D-glucosamine (PNAG) was registered by Enzyme-linked lectinosorbent assay (ELLA) using lectins with appropriate sugar specificities. Cell and biofilm morphology were examined by scanning and transmission electron microscopy after negative staining. Results: Motility of most of the strains was stimulated by SMP-E. Synthesis of cellulose as well as curli formation were not registrated in any of the strains. Co-aggregation was stimulated by SMP-E in biofilm formers but also in two of the non-formers. Individual *E. coli* strains showed different response to SMP-E regarding the production of the two exopolysaccharides. In one of the strains, expression of F pili was stimulated, and another formed filamentous forms. Conclusion: Most of the biofilm-related phenotypes of *E. coli* K-12 strains were stimulated by SMP-E. However the strain-to-strain differences found indicate different surface colonization strategies.

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## GAM19

# TWO POLYSACCHARIDE ANTIGENS ASSOCIATED WITH THE SURFACE OF *ESCHERICHIA COLI* O157: H- AND THEIR IMPACT IN ITERACTIONS WITH THE HOST

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We have previously reported on growth temperature-related changes in the surface of *E. coli* O157:H- which were expectedly due to the presence two different polysaccharides isolated, by the hot phenol-water procedure, from the phenol (Ph) and water(W) phases. This study aims comparative analysis of the two polysaccharide-rich crude fractions. NMR spectroscopy identified the presence of the O157 antigen in the phenol-phase preparation, while the water-phase contained polysaccharides with sugar composition characteristic of colanic acid and the enterobacterial commin antigen. ELISA test was performed with two diagnostic antisera developed against *E. coli* O157:H- and *E. coli* O157:H7, and the test confirmed high level of binding to the Ph sample, and insignificamt – to the W sample. To check the putative role of the two fractions in phagocytosis, FITC-labeled latex spheres were loaded with each of the samples and phagocytosis by human peripheral blood neutrophils was performed and examined by flow cytometry. Engulfment of the fluorescent spheres appeared significant only with the W-polysaccharide sample. These results suggested that interaction of individual bacterial cells with host sera or phagocytes may vary with the degree of surface exposure of the different polysaccharide antigens. Further experiments were directed to check for possible changes in the antigens state of exposure due to growth of the strain at two different temperatures: 20°C and 37°C. Since for the time being we were unable to find specific antibody for the enterobacterial common antigen and/or colanic acid, the experiments addressed the state of exposure of the O157 antigen. A whole-cell ELISA test was developed, and it showed significantly higher immunoreactivity of the bacteria grown at 20°C. Immunofluorescence confirmed these differences, and the O157 antigen appeared least accessible on

bacteria grown at 37°C. Finally, we examined the resistance to normal human serum. While the strain was characterized by generally high serum sensitivity, survival cells were more abundant at 37°C growth. In summary, the results indicate different role of the surface polysaccharides in the host-invader interplay that needs examination in further detail.

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## GAM20

## SUPEROXIDE DISMUTASE ENZYMES RESPIRATORY YEASTS *PICHIA PINI*

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Superoxide dismutase (EC: 1.15.1.1.) enzyme activity was investigated during batch-wise cultivation of *Pichia pini* 77-1 yeast strain on mineral nutrient medium containing glucose, glycerol or methanol as sole carbon source. Higher enzyme activity was determined in the methanol grown culture - 19.39 U $mg^{-1}$, followed by that in glycerol and glucose grown cells respectively: 12.89 U $mg^{-1}$ and 8.8 U $mg^{-1}$. Electrophoretical analysis (native PAGE) revealed presence of a band corresponding to KCN sensitive Cu/Zn SOD with Rm 0.27 and two Mn SOD isoenzymes (Rm = 0.47 and 0.34) in all cell-free extract samples. Moreover, a third Mn SOD band with Rm 0.12 was observed in the methanol grown culture, suggesting appearance of additional form of this enzyme under the tested conditions. Furthermore, the influence of elevated temperatures and β mercaptoethanol on the enzyme activity was investigated. Time-dependence of the enzyme activity was monitored during incubation at 50˚C and 75˚C or treatment with 30, 50 and 100 mM β mercaptoethanol. The enzyme forms visualized were stable and did not disappear after 1 min at 75°C. The band with Rm 0.34 corresponding to Mn SOD enzyme remained in all yeast samples cultivated on methanol, glucose and glycerol, after heating at 75°C for 10 minutes. The band with Rm 0.47 (Mn SOD enzyme) was stable after heating at 75°C for 1 minute and the third Mn SOD enzyme with Rm 0.12 (characteristic of methanol culture) disappeared at 50°C / 1 minute. All Mn

SOD isoenzymes were inhibited by 30 mM β mercaptoethanol. Cu/Zn SOD retained in all samples, incubated with 30, 50 and 100 mM β mercaptoethanol. Moreover, the performed spectrophotometrical analysis of the total SOD activity showed that about 28% of the total enzyme activity remained after treatment at 75°C for 10 minutes of the methanol, glucose and glycerol grown cultures and average 11.21 % after treatment with100 mM β mercaptoethanol.

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„Подкрепа за развитието на докторанти, пост-докторанти, специализанти и млади учени"

## GAM21

## EFFECTS OF EXTRACTS FROM BULGARIAN MEDICINAL PLANTS ON BIOFILM FORMATION BY UPEC CLINICAL ISOLATES

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Bacteria grown as biofilm cause serious trouble in medical practice as a source of both contamination of indwelling medicinal devices, and nosocomial infections. Due to a more rapid development of antibiotic resistance during sessile growth, if compared with planktonic microorganisms, anti-biofilm strategies address the search of substances that may suppress biofilm without killing the microorganisms themselves. This report presents the results of a screening test of plant substances for anti-biofilm capacity. Initially, 28 strains of clinically isolated uropathogenic *E. coli* (UPEC) were tested for biofilm formation. Three of them form sufficient biofilm and were chosen to further check anti-biofilm activities. Antibiotic resistance of these was determined by the disk-diffusion assay. The effects of 14 extracts from 4 medicinal plants in different organic solvents were tested. The dried extracts were dissolved as stocks in ethanol. Disk diffusion assay with different amounts of the extracts showed no antibacterial activety against the selected strains. Biofilm growth

was examined by the crystal violet assay after growth for 24 h in M63 medium alone or supplemented with 10 μg/ml of the dried extracts. In one of the strains, *E. coli* PU-1, biofilm formation was very sensitive to the treatments, and all extracts significantly suppressed sessile growth. *E. coli* PU-13 was less sensitive with only 30% of the extracts effective. Surprisingly, in strain *E. coli* PU-19 all treatments stimulated instead of suppressing biofilm growth. This strain is multidrug-resistant but protection by efflux pumps alone could not account for this effect. A parallel examination of growth curves showed suppression of its summary (plankton plus biofilm) growth. Thus, biofilm stimulation can be considered as a survival strategy in *E. coli* PU-19.

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## GAM22

## CHANGES IN HeLa CELLS LOCALIZATION OF THE MICROTUBULE-RELATED TRANSPORT ACTIVATOR VDP UPON INTERACTION WITH *E. COLI* O157:H-

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*E. coli* O157 is known to remodel the actin cytoskeleton of host cells via its Type III secretion mechanisms. Modulation of actin location is accompanied with the formation of attaching and effacing lesions of the eukaryotes, and localized adhesion of the bacteria. Here we address the question whether actin re-arrangements are accompanied with alterations of microtubule-related processes of intracellular transport. For this reason, the localization of VDP, a microtubule-associated ERGIC protein responsible for intracellular traffic of vesicles, was chosen as a marker. E. coli O157:H- was cultivated in TSB 18 h at 37°C. HeLa cells monolayers were inoculated with a final concentration of $10^6$ bacterial cells per ml and incubated for 1 h at 37°C, 5% $CO_2$. Slides were fixed and Giemsa stained, or double labelled with primery anti-VDP for ERGIC membrane activator and FITC-conjugated secondary antibodies. Giemsa-stained samples were observed by light microscopy. It confirmed the localized adhesion of the bacteria indicative of actin re-arrangements. CLSM examinations revealed alterations in the localization of VDP. In the presence of bac-

teria, the HeLa cells were significantly more intensively labelled than control cells incubated in the absence of the pathogen, and VDP patchy localization near the extracellular membrane was observed. This implies that the strain may cause alterations in microtubule-related intracellular transport.

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## GAM23

## CALGEVAX (BCG) IN TREATMENT OF RECURRENT RESPIRATORY PAPILLOMATOSIS

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Although there is a growing interest among clinicians in using immunotherapy as an adjunct to surgery, chemotherapy and/or radiotherapy in the treatment of cancer, the role of Bacillus Calmette-Guérin (BCG) has not been established in the prevention of recurrence for laryngeal cancer.

Preliminary results on the effect of BCG in the treatment of laryngeal cancer are reported. This study focused on the Bulgarian product CALGEVAX (BB-NCIPD Ltd.) specially designed for treatment of cancer.

The main characteristic of the recurrent respiratory papillomatosis - a disease with DNA viral etiology -is the recurrent proliferation of benign squamous papillomas.

The patients were divided into three groups: I: Surgery + consequent $CO_2$ laser therapy (15 patients), II: Surgery +α-Interferon application by a scheme (15 patients), and III: Surgery + BCG immunotherapy by percutaneous route (a schedule with BCG application every 40 days) (10 patients). The dose applied is $2.56 \times 10^8$ CFU. The period of observation is 10 months.

The preliminary results demonstrated that the BCG immunotherapy is superior to both: surgery + laser therapy and surgery + treatment with α-Interferon. The results show that the BCG therapy is tolerated very well. No local and/or systemic adverse effects after immunotherapy with Calgevax in the dose and schedule applied were encountered. Favorable results were seen in all patients in term of recurrences.

Currently there is ongoing research aimed at optimal immunotherapy with Calgevax of this insidious disease.

## GAM24

# PURIFICATION AND PARTIAL CHARACTERIZATION OF COLD-ACTIVE ENZYME SUPEROXIDE DISMUTASE

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The enzyme superoxide dismutases (SOD) provide a primary line of the antioxidant defence. This enzyme naturally present in all aerobic cells catalyzes the dismutation of the highly reactive superoxide radical anion to hydrogen peroxide and molecular oxygen. Cold-active SOD can be useful in cryosurgery (liver transplantation), cryopreservation processes, artificial insemenetion and in *in vitro* fertilization. It will also be very important antioxidant in all cosmetic formulations, to promote younger looking skin, to protect against damaging effects of UV light (photoaging), environmental pollutants etc.

The fungal strain *Aspergillus glaucus* 363, isolated from soil samples taken from Livingstone Island, maritime Antarctica, produced cold-active superoxide dismutase (SOD). Effective method for isolation of the intracellular enzyme was developed. The cell-free extract was clarified throught cellite and Millipore's device Pellicon XL Durapore 0.1, concentrated and fractionated by ultrafiltration with Pellicon XL 50 and Pellicon XL 10 to receive two main fractions: above 50 kDa and between 10 to 50 kDa. These fractions were subjected for purification with anionic chromatography on Q-sepharose, and next step by hydrophobic chromatography on Phenyl-sepharose. Two components of SOD were obtained - major with MW about 30 kDa and high activity (3500-4000 U/mg) and minor component with lower activity (about 500 U/mg) and stability and MW about 90 kDa, which were observed on specific PAGE (according to Davis).

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## GAM25

# PREPARATION, PERFORMANCE AND APPLICATION OF *ASPERGILLUS NIGER* B 03 ENDO-XYLANASE IMMOBILIZED ON THE SMART POLYMERS EUDRAGIT S-100 AND L-100

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*Aspergillus niger* B 03 endo-xylanase was immobilized on the smart polymer Eudragit S- and L-100. Solubility of the two types Eudragit was determined by changing pH from 2 to 7 and measuring the % T at 600 nm. By varing the amount of added enzyme, maximum immobilization and activity yield was determined – 67.6 % and 44.7 %for Eudragit L-100 and 60.5 % and 59.7 % for Eudragit S-100 . The degree of immobilized xylanase was determined also using different concentration of the polymer. Maximum immobilization yield achieved with 2 % polymer concentration was 88.6% for Eudragit S-100 and 50.4 % for Eudragit L-100. The basic biochemical characteristics of the immobilized xylanase were determined. Immobilized enzyme exhibited maximum activity at pH 4 – 4.5 and at temperature 50 – 55 ºC . Stability at 40 and 50 ºC and reusability at 40 ºC were also investigated. Kinetic parameters Km and Vmax, and the amount of reducing sugars obtained after enzymatic hydrolysis of oat spelt and birchwood xylan were determined.

## GAM26

# BIOCHEMICAL CHARACTERIZATION OF RECOMBINANT STRAINS OF *LACTOBACILLUS BA68, LACTOBACILLUS RB44, LACTOBACILLUS RD13*

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The biochemical profiles of the recombinant strains *Lactobacillus BA68, Lactobacillus RB44, Lactobacillus RD13*, obtained by hybridization of *Lactobacillus acidophilus 2 ($Str^R$ $Neo^S$)* and *Bifidobacterium bifidum $L_1$ ($Str^S$ $Neo^R$)*

have been examined. Their ability to transform 49 carbon sources included in the API 50 CHL (BioMerieux, France) has been determined.

Significant differences between the recombinants and the parental cultures have been revealed. Modification of some regulatory mechanisms with phenotypic manifestations such as loss of ability to degrade some substrates included in the kit or obtaining the capacity to utilize maltose, inulin, to hydrolyse aesculin and others, have been observed.

It has been shown that through genetic recombination a number of properties of the hybrids compared with the properties of their parental cultures may be amended.

## GAM27

## ANTIOXIDANT ACTIVITIES AND BIOACTIVE COMPOUNDS IN EXTRACTS OF *SALVIA TOMENTOSA* MILL. PLANT AND CELL SUSPENSION CULTURES

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The plants from *Lamiaceae* family, especially the members, belonging to the genus *Salvia*, are one of the richest sources of natural extracts, widely used as active ingredients in many cosmetic and aromatic products. The balsamic sage (*Salvia tomentosa* Mill.) is an economic important *Salvia* species, since it is widely used for tea preparation and as flavoring agent in perfumery and cosmetics. This plant, known as "Edrocvetna kakula", growing in very limited populations in Bulgaria, and in 2007 it was added to the list of species which collection was prohibited for commercial purposes, but was allowed for personal use (The Order no. RD-71/07.02.2007).

In the present study, we present the effective method for obtaining of cell suspension cultures of *S. tomentosa* Mill plant. The antioxidant activities of the extracts from intact plant and the obtained *in vitro* suspensions were compared and discussed in the context of their phenolics, flavonoids, ursolic and oleanolic acids content.

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ian Ministry of Education and Science (project ДМУ – 02/9, 2009).

## GAM28

### OPTIMIZATION OF THE COMPOSITION OF THE MEDIUM FOR OBTAINING LACTIC ACID WITH *LACTOBACILLUS HELVETICUS SSP. RHAMNOSUS* NBIMCC 507

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Microorganisms need an appropriate medium and cultivation conditions for their development and the synthesis of metabolites. The medium for obtaining lactic acid by free cells of *Lactobacillus helveticus ssp.rhamnosus* NBIMCC 507 has been modeled and optimized. The managing factors and the limits of their variation have been determined through single factor experiments and statistical analysis. Modeling and optimizing of the composition of the medium is done by orthogonal central composition plan. Based on the conducted optimization the content of the medium for obtaining lactic acid has been obtained (g/dm$^3$): glucose - 70.4; meat extract - 17:07; yeast extract - 10.9; $CH_3COONa$ - 10; $K_2HPO_4$ - 0.25; $KH_2PO_4$ - 0.25; $MgSO_4.7H_2O$ - 0.1; $MnSO_4.7H_2O$ - 0.05; $FeSO_4$ - 0.05. The maximum specific growth rate of the lactic acid bacteria during cultivation in a laboratory bioreactor ($\mu_m = 0.637h^{-1}$) and the constants of utilization of glucose and the accumulation of lactic acid in the cultural medium have been determined.

## GAM29

### ANAEROBIC DIGESTION OF CELLULOSE CONTAINING WASTES IN MESOPHILLIC CONDITIONS

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Batch laboratory experiments for anaerobic digestion of cellulose containing wastes in mesophillic conditions (t=34°C ± 1°C) have been performed. Two

different media (CM3 and Imshenetskii medium) for the cultivation of cellulolytic microorganisms have been used. The increase in the cellulose amount has been obtained by adding filter paper and microcrystalline cellulose. During the experiments, the following variables have been measured: VFA concentration (Ripley method), reducing sugar (Miller method), dry weight, pH and biogas production. The population dynamics of the cellulolytic microorganisms has been determined using serial dilutions and McCready tables. The population dynamics of the heterotrophic microorganisms has also been determined.

The results show that the dry weight decrease with 2/3 in the both media, reaching 30 % from the initial amount. The quantity of the generated monosaccharides (like reducing sugar) on the CM3 medium exceeds those of the Imshenetskii medium. Larger quantity of VFA-s have been formed on Imshenetskii medium, but their adoption with biogas production is better on the CM3 medium. The population dynamics of cellulolytic microorganisms in the both media has been approximately equal. The preference is for CM3 medium, which ought to be optimized.

## GAM30

## BIOREACTOR PRODUCTIONS OF GALANTHAMINE BY *LEUCOJUM AESTIVUM* L. SHOOT CULTURE. A COMPARATIVE STUDY.

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Galanthamine is widely used in medicine for the treatment of Alzheimer‘s disease, poliomyelitis and other neurological diseases. Shortage of natural resources for its obtaining during the last decade leaded to necessity of development of an alternative approaches for its production.

The process of galanthamine and related alkaloids production by *Leucojum aestivum* L. shoot culture in a temporary immersion system and bubble column bioreactor with internal sections were studied. It was established that the both cultivation systems are appropriate for shoot culture cultivation. After optimization of the environmental parameters of both cultivation systems it was established that the highest galanthamine yields (1.68mg/L) were achieved during the cultivation of *Leucojum aestivum* L. shoot in the bubble column

bioreactor at flow rate of inlet air 18 L.(L.h)$^{-1}$ and temperature 22°C. Beside the galanthamione the invitro culture synthesized (1.40mg/L) norgalanthamine and (8.32mg/L) lycorine. The last both alkaloids are well with their high biological activities, as well.

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## GAM31

# DECOLORIZATION OF DIFFERENT CLASSES OF DYES BY IMMOBILIZED MYCELIA OF *TRAMETES VERSICOLOR*

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Dyes belonging to the mono- and di-azo as well as anthraquinonic and reactive classes, chosen among the most utilized in textile applications, were employed for a comparative decolorization study using the white rot fungus *Trametes versicolor*, immobilized in different supports. The most suitable method for immobilization (to achieve maximum decolorization) was found to be self aggregation followed with cross linking by glutaraldehyde. The immobilized fungus was found to decolorize up to 75% **Phenol Red** and completely **Orange II, Congo Red, Reactive Violet 12, Brilliant Blue R, Reactive Blue 4** and **Indigo Carmine** at an initial dye concentration of 125 g/l, within 48 h of incubation in a "batch" reactor type, using 10 % (m/v) biocatalizator concentration. The increasing of biocatalizator concentration up to 30% and 60% leads to completely decolorization in a very short period of time (4-5 hours). The operational stability of immobilized preparations was found to be more than 36 batches in case of Congo Red and about 5-9 batches in case of other dyes. During decolorization in liquid medium (only water), laccase activity was detected in culture filtrate. Dye-decolorizing activity of the immobilized culture was found to be associated with the processes of biodegradation, bio-oxidation and biosorption.

## GAM32

# BULGARIAN REFERENCE REAGENT FOR BCG VACCINE: VIABILTY EVALUATED BY THE BIOLUMINESCENT ATP-ASSAY AND VIABLE COUNT METHOD

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The first WHO Reference Reagents for BCG vaccines of three different substrains – Danish 1331, Tokyo 172-1 and Russian BCG-I, have been developed and adopted by the WHO ECBS in 2009. These preparations cover all substrains used for production of BCG vaccines currently prequalified by the WHO. It is investigated the BCG vaccine, Bulgarian Lot 254-2 produced from Sofia SL 222, itself originating from the Russian BCG-I substrain. All three substrains are evaluated in an international collaborative study of eleven laboratories (WHO, ECBS, K.Markey, Mei M.Ho et al., 2009) using two viability assays (cultural viable count and modified ATP assay, based on the firefly luciferase) and mPCR as an identity assay.

A study, defining the content of the Bulgarian Reference Reagent (RR) candidate in terms of viability (both ATP and CFU content) is reported.

The inclusion of a modified ATP method according to a protocol provided for the purposes of the collaborative study allowed checking the suitability of this rapid and sensitive method as an alternative method of the cultural viable count assay for viability.

For the cultural viable count assay the routine in-house method (BCG Laboratory, BulBio-NCIPD) based on the principles of the WHO recommendations (WHO)/TB/Technical Guide/77.9) is applied.

The ATP content of the RR for BCG Lot 254-2 estimated in the Bul Bio laboratory is mean 4.11±0.88 ng per ampoule. In the collaborative study this content is 7.52±1.48 ng ATP per ampoule (estimated from seven data sets). As the ATP conditions varied among all participating laboratories, the distribution of the mean ATP content obtained from the participants is wide (the expanded uncertainty is 95%, the confidence is 0 to 16.8).

The mean CFU determined at the Bul Bio laboratory of the Bulgarian candidate is 3.07±0.29 million per ampoule. In the collaborative study the estimated CFU (ten data sets) confirm the expected variable results due to the different

culture media and methodologies used in the laboratories. The mean CFU is 3.39±0.50 million per ampoule. The expanded uncertainty (95% confidence) varies from 0.95 to 5.83 million.

The results demonstrate the suitability of the Bulgarian Lot 254-2 for use as a Reference Reagent for the BCG vaccine and as a comparator for viability assays, residual virulence/local reactogenicity assays and protection assay in animal models.

## GAM33

# ANAEROBIC DIGESTION OF CATTLE DUNG IN BIOPROCESS SYSTEM WITH FIXED FILM

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Biogas production from cattle dung in a new biofilm reactor has been realized in laboratory living model. As carrier has been used inert plastic mesh supported by sheets of material, resistant to aggression of the liquid media and microorganisms. The carrier and the support have been assembled together to a packed bed forming the biofilm reaction zone. There the biogas has been produced by means of anaerobic microbial society, spontaneously formed self organized biofilm system on the packed bed-carrier. The created biofilm reactor can be related to the class of the submerged packed bed with internal loop, ensured a suitable circulation rate by a special mixing device and appropriate distribution system. The high degree of airproof has been provided by magnetic set-up coupling indirectly the bioreactor stirrer with the electromotor and in such away avoiding the probability of oxygen penetration into the reaction zone.

The comparative studies with a well known bioreactor with suspended culture for biogas production have shown that the new submerged packed bed biofilm reactor has been with two to three times higher intensity. The biofilm reactor created is applicable to function under different conditions – in continuous as well as in batch or in fed-batch regimes. It has been found that the new biofilm reactor can serve also as laboratory tool for biokinetics investigations with real substrates as well as for investigation on the biofilm formation and functioning during long periods allowing observation of the biofilm behavior during the consecutive phase of its living cycle.

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## GAM34

# COMPARATIVE ASSESSMENT OF THE ANTIOXIDANT CAPACITY OF *LACTOCOCCUS LACTIS SSP. LACTIS* AND *PROPIONIBACTERIUM FREUDENREICHII SSP. SHERMANII*

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To increase the health effect of food antioxidant substances or microorganisms that are potential sources of substances with antioxidant activity and have the ability to disarm free radicals are included In food.

The antioxidant activity of *Lactococcus lactis subsp.lactis* NBIMCC 3707*, Propionibacterium freudenreichii subsp.shermanii* NBIMCC 327 and *Propionibacterium freudenreichii subsp.shermanii* NBIMCC 328 has been established with the use of the ORAC method. It has been shown that lactobacilli form antioxidative capacity that is higher in the cultural medium (16.96 μmol TE / logN) in comparison with that of the lysed cells (1.25 μmol TE / logN).

The ability of propionic acid bacteria to form and secrete metabolites with antioxidant activity (10.34 μmol TE/$cm^3$) and to disarm peroxide radicals has been shown. Their catalase, peroxidase and superoxiddismutase activity has been defined.

It has been shown that superoxiddismutase is strain specific and is influenced by the time of cultivation when it comes to propionic acid bacteria. 48 hour cultures of *Propionibacterium freudenreichii subsp.shermanii* NBIMCC 327 have 80% higher superoxiddismutase activity in comparison with 24 hour cultures.

## GAM35

# COMPARATIVE STUDY OF OBTAINING OF LACTIC ACID WITH FREE AND IMMOBILIZED CELLS OF *LACTOBACILLUS CASEI SSP. RHAMNOSUS* NBIMCC 1013

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In recent years research on the application of immobilized microbial cells through their inclusion in various gel structures is expanding.The obtaining of lactic acid by free and immobilized cells of *Lactobacillus casei ssp.rhamnosus* NBIMCC 1013 has been examined. Lactic acid bacteria are immobilized in Ca-alginate. Periodic fermentation processes in a laboratory bioreactor with agitation and in a column bioreactor with thick layer of immobilized biocatalysts have been carried out. The main process parameters such as pH, Eh, concentration of lactic acid have been monitored. Differences in the physiological status of the free and immobilized cells due to the presence of diffusion barrier formed by the matrix of the medium, have been observed.

## GAM36

# OBTAINING LACTIC ACID WITH IMMOBILIZED \LACTIC ACID BACTERIA

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The obtaining of lactic acid with immobilized cells is a recent trend. The usage of various matrices increases the concentration of cells in the working volume of the bioreactor and the yield of the final product. Immobilization of the lactic acid bacteria *Lactobacillus casei ssp.rhamnosus* NBIMCC 1013 and *Lactobacillus helveticus ssp. rhamnosus* NBIMCC 507 in the Ca-alginate and k-carrageenan has been carried out. The dynamics of the growth of the cells in the gel matrix for 60 h at cultivation temperature of 37 ºC have been monitored. It has been shown that immobilized lactic acid bacteria retain their activity during the fermentation process. It was found that cell immobilization reduces the fermentation time during cultivation in laboratory column bioreac-

tor with a thick layer reaching a threefold higher concentration of lactic acid in the medium - 65 g/dm$^3$ compared to free cells. It has been shown that the cells embedded in the gel structures increase their number to $10^{16}$ cfu/g.

## GAM37

# SELECTED STRAINS WITH INTESTINAL ORIGIN AND FROM BULGARIAN YOGHURT INHIBIT TGF-BETA AND INTERLEUKIN-8 SECRETION FROM EPITHEAL CELLS, AND INDUCE SECRETION OF INTERLEUKIN-10 FROM MACROPHAGES

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Intestinal epithelium is capable of releasing some proinflammatory cytokines such as IL-8 and TGF-beta especially when stimulated by cytokines like TNF-alpha. Some *Lactobacillus* strains could modulate intestinal mucosal immune response playing anti-inflammatory role in the intestinal immune signaling. The aim of the present study was to evaluate the effect of *Lactobacillus* strains on IL-8 and TGF-beta secretion in intestinal epithelia with and without stimulation by TNF-alpha. A large number of strains with yoghurt and intestinal origin were evaluated in their ability to reduce the production of IL-8 and TGF-beta from Caco-2 cells. The strains demonstrated the highest reduction of these proinflammatory cytokines were *B. bifidum* Bif8, *L. gasseri* G8, *E. faecalis* E2, *L. bulgaricus* B67, and *S. thermophilus* T43. It should be noted the yoghurt origin of the latest two strains. In order to complete the information about anti-inflammatory potential of the strains, they were evaluated in induction of IL-10 using macrophage cell line model U-937. The strains *B. bifidum* Bif8, *L. gasseri* G8, *E. faecalis* E2 induced the production of IL-10 and could be used for preparation of yoghurts for clinical trials of their anti-inflammatory effect.

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## GAM38

# DEVELOPMENT OF STRAIN DISCRIMINATIVE AFLP FOR LACTOBACILLI WITH INTESTINAL ORIGIN

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Several health-promoting effects have been attributed to intestinal lactobacilli as a part of the normal intestinal microbiota. Because of the probiotic properties are strain-specific, the use of reliable and discriminative molecular methods is very important. We isolated a total of forty nine strains of lactic acid bacteria from the faeces of healthy donors. The species in that group were determined as *L. plantarum* (11 strains), *L. casei* (11 strains), *L. rhamnosus* (7 strains), *L. fermentum* (7 strains), *L. gasseri* (6 strains), *L. delbrueckii ssp. lactis* (4 strains), *L. salivarius* (2 strains), and *L. acidophilus* (1 strain). Genotyping at strain level was performed using pulsed field gel electrophoresis (PFGE) with endonucleases *ApaI* and *XhoI* and amplified fragment length polymorphism (AFLP) with enzymes *XhoI* and *TaqI*. The goal of the present work was to develop AFLP genotyping for intestinal lactobacilli with increased discriminatory power and reproducibility. It is based on initial cleavage of DNA-s with enzyme couple Xho I and Taq I, specially designed adapters, preselective and selective PCR primers. The developed AFLP analysis proved to be at least as discriminative as PFGE. AFLP could differentiate strains with the same PFGE profiles. Therefore, AFLP successfully could replace the labor consuming PFGE. The specially developed AFLP and PFGE proved very high potential to evaluate the strain diversity of *Lactobacillus* spp. with human origin.

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**GAM39**

# CHARACTERIZATION OF ACTIVITY AND EXPRESSION OF VIRULENCE FACTORS OF ENETEROCOCCI ISOLATED FROM TRADITIONAL BULGARIAN CHEESES

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Enterococcal factors that contribute to pathogenesis have been identified and include cytolysin, aggregation substance, adhesins and hydrolyticenzymes. Among these enzymes is gelatinase, an extracellular zinc metalloprotease that has been shown to potentially contribute to *Enterococcus faecalis* virulence.

75 isolates from traditionally fermented Bulgarian cheeses, identified as *Enterococcus faecalis, Enterococcus faecium and Enterococcus durans* were screened for their capacity to produce gelatinase and for the presence of the genes. We studied the presence of nine virulence genes (gelE , hyl , asa1, esp, cylA , ace , efaA , vanA and vanB) in our cheese Enterococcus isolates. We detected the gelE gene in all the E. faecalis isolates but not in *E.durans and E.faecium*. The remaining genes screened - cylA , hyl , vanA and vanB, were not detected. Results demonstrated that the virulence factor gelatinase is disseminated among the genus.

The silent behavior of gelE was only observed in *E. faecalis* , but not in E. durans or *E.faecium*, suggesting different modulation mechanisms of gelatinase activity in these three species. Overall, these findings reopen the issue of food safety of enterococci and reinforce the need to further study the mechanisms responsible for triggering the virulence factor gelatinase in non- pathogenic enterococcal environmental isolates.

## GAM40

# ASSIMILATION OF CHOLESTEROL BY LACTIC ACID BACTERIA

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Serum cholesterol in humans is generally a risk factor correlated with the development of coronary heart disease. Cardiovascular diasease is the most important cause of death in the westernized countries and it is strongly associated with hypercholesterolemia. Decreasing serum cholesterol is, therefore, very important to prevent cardiovascular disease. Cholesterol – lowering effect of lactic acid bacteria ( LAB: *Streptococcus, Lactobacillus and Bifidobacterium* ) is well know. In the recent years, a particular accent was placed on fermented products with specific microorganisms to which nutritional, dietetics and therapeutics properties were attributed. We investigated LAB of **LBB collection of LB Bulgaricum** for their ability to remove cholesterol from laboratory media during growth in presence of cholesterol and bile salts.We using for determination of cholesterol in medium *o-phthalaldehyde method*. The o-phthalaldechyde determination is three times more sensitive than the $FeCl_3$ determination. The cholesterol removal relate to the ability of the cultures to deconjugate bile salts. The suplemented the culture medium for determination of cholesterol reduction was done with Lipids Cholesterol Rich ( Sigma, L- 4646). This method is rapid and sensitive. Fifty strains were tested for their ability to remove cholesterol from growth medium (MRS, $M_{17}$, Elliker broth) supplemented with 0,3% Oxgall. The uptake of cholesterol occurred only when the cultures were growing in the presence of bile under anaerobic conditions. The examined strains were as follows:11of these strains were of genus *Lactobacillus* where 6six are *Lb. bulgaricus*, four *Lb. helveticus* and one *Lb. lactis*. Eighteen of strains belong to species *Str.thermophilus*. Fifteen of strains were *Lactococcus lactis*. The amount of cholesterol removed during 20h of growth at $37^0$C and $30^o$C revealeated significant variations between different lactic acid bacteria. Cholesterol assimilation as determined by the difference in cholesterol content in the medium before and after the incubation period showed that all lactobacilli strains were able assimilated cholesterol at

varying levels ranging from 0,8 – 66 μg/ml, streptococci strains assimilated cholesterol at varying levels ranging from 2- 49 μg/ml, and lactococci from 7 to 90 μg/ml. *Lactococcus lactis l598* decreased cholesterol concentration to 90% in the growth media. All strains of the *Streptococccus* reduced cholesterol in medium under 50%. *Lactococcus* show the strongest hypocholesterolemic effect . These activity is weak by genus *Lactobacillus* . The strains of *Streptococcus* showed the most weak hypocholesterolemic effect .The ability to assimilate cholesterol in vitro of some strains of genus *Lactobacillus* and genus *Lactococcus* may be promising candidate strains for use as probiotics , a dietary adjunct to lower serum cholesterol in vivo.

## GAM41

## PHYTOPATHOPGENIC BACTERIA OF GENUS *BURKHOLDERIA* IN BULGARIA

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The aim of this work is to study the species and intraspecies diversity of bacteria of genus *Burkholderia* isolated from onion bulbs in Bulgaria.

Twenty two strains were isolated from infected plant material. The type cultures of *Burkholderia gladioli* pv. *gladioli, Burkholderia gladioli* pv. *alliicola, Burkholderia cepacia,* as well as clinic isolates of *B. cepacia* were used as controls. The identification of the pathogenic strains was performed by BIOLOG system and three species were established - *B. cepacia*, *B. gladioli* and *B. pyrrocinia*. The intraspecies diversity was studied on the basis of the metabolite profile of the strains, obtained by BIOLOG using cluster analysis. The strains were separated into seven clusters, which showed the species and intraspecies diversity, greater among the strains of *B. gladioli*. The clinic isolates of *B. cepacia* distinguished from the phytopathogenic ones. The sensibility to 14 antibiotics from different groups was investigated and after clustering the strains were separated into four clusters at 80% similarity. The strains identified as *B. cepacia* and *B. pyrrocinia* formed one cluster. The relationships of the Bulgarian strains, as well as clinic ones and type cultures *B. cepacia, B. gladioli* pv. *allicola, B. gladioli* pv. *gladioli* with several microorganisms were examined. The strains identified as *B. cepacia* and *B. pyrrocinia* possessed the broadest range of antimicrobial activity. The diversity among the populations

of *Burkholderia* strains was studied by molecular approach. RFLP – analysis was carried out with 16S rDNA amplified fragment by the pair of universal primers (9f and 1542r) and endonucleases *Alu*I, *Tas*I and *Taq*I. The selected enzymes discriminated *B. cepacia* from *B. gladioli*, as well as the two pathovars of *B. gladioli*. The enzyme *Alu*I revealed polymorphism among the *B. gladioli* pv *gladioli* strains. This analysis did not confirmed species belonging of the strains identified as *B. pyrrocinia* by BIOLOG. The applied polyphasic approach allowed the differentiation between *B. cepacia* and *B. gladioli* and the two pathovars of *B. gladioli.*

## GAM42

## Cu (II) STRESS RESPONSE OF GROWING *ASPERGILLUS NIGER* CELLS

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AIMS: To investigate the relationship between the growth, participation of superoxide dismutase (SOD), catalase (CAT), cellular acid phosphatase (AP) and Cu (II) uptake in the protection of *Aspergillus niger* against Cu (II) stress.

RESULTS: The stress response of the fungal cells, under conditions of high Cu (II) concentrations, was investigated by determining the biomass formation, SOD, CAT and AP activities, and heavy metal uptake during the growth of *Asp. niger*. The exposure to high Cu (II) concentrations induced an increase of the enzyme activities, heavy metal ions uptake and decrease of biomass formation.

COCLUSION: The results obtained indicated that the resistance of *Asp. niger* to Cu (II) was correlated with heavy metal uptake, enhanced activity of AP, reactive oxygen species generation in the cells and the efficiency of antioxidative defense system.

SIGNIFICANT OF THE STUDY: These data could offer useful information when creating new strategies for bioremediation of heavy metals with the participation of fungi.

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**GAM43**

# THERMOPHYSICAL AND IR SPECTRAL CHARACTERISTICS OF AN EXOPOLYSACCHARIDE SYNTHESIZED BY THE PSYCHROPHILIC YEAST ISOLATE 102

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A new producer of exopolysaccharide from Antarctic yeast was selected. The microbial carbohydrate was a heteropolysaccharide of the following monosaccharide composition: arabinose: 59.6%, mannose: 16.4%, glucose: 13.2%, galactose: 5.6% and rhamnose: 3.18%. This work presents the glass transition results and the enthalpy of two endothermic effects obtained from DSC measurements of an exopolysaccharides synthesized from Isolate 102. The experimental data from the DSC measurements were compared to those obtained from DTA and TGA which resulted in identifying three endothermic effects with maxima displaced towards the lower temperature readings. The IR spectra of the exopolysaccharides under study revealed absorption bands characteristic of natural polysaccharides.

The study was supported by European Social Fund, Operational Programme "Human Resources Development", Contract BG051PO001-3.3.04/32.

## GAM44

# PCR DETECTION AND 16S RDNA SEQUENCE ANALYSIS OF DIFFERENT *ACIDITHIOBACILLUS FERROOXIDANS* ISOLATES

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This study aimed to evaluate rapid and specific PCR assay for identification of *Aciditiobacillus ferrooxidans* strains based on amplification of 16S rDNA sequence. Bacteria belonging to this species appear to be present in a wide range of environments, especially in sights where pH is low, such as mining areas, hot sulphur springs and bioleaching operations, which determines high morphological, biochemical and genetic polymorphism. In the present work is used total genomic DNA isolated from ten strains *A. ferrooxidans*, originating from different niches. 16S rDNA PCR methodology is used to amplify a 980 bp fragment from each strain. DNA sequence analysis and bioinformatics interpretations reviled significantly high genetic identity in this fragment between all tested strains, as well with the type strain. Sequence alignment of the fragments demonstrates the presence of highly homogeneous region of 380 bp, which could be successfully applied for further molecular investigations.

## GAM45

# BIODIVERSITY OF IRON BACTERIA IN NATURAL HABITATS IN VITOSHA MOUNTAIN

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Neutrophilic iron bacteria are important environmental factor which could be used as indicator for organic pollution because their ability to grow at low concentrations of organic carbon. On the same time some ecological problems can arise because they are able to oxidize $Fe^{2+}$ to $Fe^{3+}$ and the pollution with insoluble iron compounds can be achieved.

Different natural habitats (streams) with visible iron pollution in Vitosha Mountain were investigated for the presence of these bacteria. Six nutrition mediums as well as specific procedures for cultivation of iron bacteria in laboratory conditions were developed.

Identification of the bacteria was done on the bases of morphological characteristics and growth ability on different media. The prevalent members of bacterial community appear to be bacteria from genera *Sphaerotilus* and *Leptotrix*. In some samples the bacteria from genus *Galionella* were found. The obtained pure cultures will be used in further nanotechnology experiments.

## GAM46

# MICROBIAL COLONIZATION AND IDENTIFICATION OF BACTERIAL STRAINS ISOLATED FROM THRACIAN VAULTS NEAR KASANLAK, BULGARIA

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Ancient Thracian vaults in Bulgaria are monuments with significant historical and cultural value. The microorganisms play an important role in the destruction of the monuments. . The monitoring of microbial communities and colonization of seven Thracian vaults, situated near to Kasanlak town, Bulgaria, is the main goal of this investigation.

Determination of the prevalent physiological groups of microbial community, their role in the processes of destruction as well as the mechanisms of their action was tested too. Eleven physiological groups considered as potential biodeteriogenes were investigated. Different morphological and biochemical tests were carried out with pure cultures, isolated from the predominant physiological groups and the taxonomic status of the isolates were achieved by the methods of the classical and molecular taxonomy. It was found that in each vault a specific microbial community was present depending on physicochemical cognitions. More than 80% of the bacterial isolates belong to the genera *Pseudomonas, Bacillus, Desulfobacter*, *Desulfovibrio and Desulfotomaculum.* The bacterial species *Arthrobacter aurescens, Kocuria halotolerance and Collinnsella aerofaciens* were found as a component of the microbial community of the two of the vaults tested.

The character of microbial colonization is in the strong correlation with the physicochemical cognitions of each vault. The temperature, humidity and oxygen were the most important factors affected the microbial colonization. Consider the negative deteriogenic effect of the active isolates, a strategy including different procedures for conservation and restoration of each monument will be proposed.

## GAM47

## LONG - TERM STUDIES ON THE MICROFLORA AND MICROBIAL PROCESSES IN A DUMP CONSISTING OF COPPER SULPHIDE ORE

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A dump consisting of about 8 600 tons of a freshly excavated rich-in-pyrite copper sulphide ore was constructed in the area near the Vlaikov Vrah copper mine, Bulgaria. The ore initially had a pH of 4.6 and contained 0.37% copper, 3.12% iron, 2.62% sulphur, 1.90% carbonates, and a highly negative net neutralization potential ( - 55 kg $CaCO_3$/t). The contact with the air and rain water stimulated the chemical and electrochemical processes of sulphide oxidation, and facilitated the initial colonization of the dump by a microbial community consisting mainly of neutrophilic sulphur-oxidizing chemolithotrophs of the genus *Thiobacillus* and some oligocarbophilic heterotrophs. The microflora of the dump and processes of bacterial oxidation of sulphides were subjects of detailed studies for a period of about 30 years. The decrease of the ore pH to less than 4 made the dump a suitable site for the growth and activity of different mesolphilic chemolithotrophic bacteria which oxidized efficiently the sulphides minerals. *Acidithiobacillus ferrooxidans*, *At. thiooxidans* and *Leptospirillum ferrooxidans* were the prevalent species of this type but some moderately thermophilic chemolithotrophs inhabited some rich-in-pyrite parts of the dump. As a result of this, the dump was, after rainfall, an intensive source of acid drainage waters containing iron, copper, aluminum and manganese as the main components. The data and conclusions from these studies were used to stimulate the processes of bacterial leaching of copper in some commercial-scale operations in Bulgaria and abroad.

**GAM48**

# BIOREMEDIATION OF WATERS POLLUTED WITH HEAVY METALS, ARSENIC AND CRUDE OIL BY MEANS OF A PASSIVE SYSTEM

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Waters with a pH in the range of 2.8 – 4.1 and polluted with heavy metals (copper, zinc, cadmium, iron, manganese), arsenic, sulphates and crude oil were treated by means of a passive system consisting of a permeable reactive multibarrier and a constructed aerobic wetland arrange in a series. The permeable multibarrier consisted of an alkalizing limestone drain and an anoxic section rich in biodegradable solid organic substrates and inhabited by sulphate-reducing bacteria and other metabolically interdependent microorganisms. The water flow rate through the multibarrier varied in the range at about 3-6 $m^3$/24 h, reflecting water residence times of about 48 to 24 h. An efficient removal of metals, arsenic and sulphates was achieved in the multibarrier, mainly due to the processes of microbial dissimilatory sulphate reduction and biosorption. The multibarreir effluents were enriched in dissolved organic compounds and in some cases still contained iron, manganese and crude oil in concentrations higher than the relevant permissible levels. The pollutants were removed in the constructed wetland which was inhabited mainly by various heterotrophic microorganisms. Iron and manganese were removed as $Fe(OH)_3$ and $MnO_2$ as a result of the prior bacterial oxidation of $Fe^{2+}$ and $Mn^{2+}$ to $Fe^{3+}$ and $Mn^{4+}$, respectively. The organic compounds, including the oil hydrocarbons, were degraded to $CO_2$ and $H_2O$ as major final products.

**GAM49**

# COMPARATIVE STUDIES ON SOME PROBIOTIC PROPERTIES OF THREE STRAINS OF *BIFIDOBACTERIUM LONGUM* FROM HUMAN ORIGIN

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In the last few years the interest in the connection between food and health increases globally. Among the diversity of functional foods, the probiotics are of special interest resulting in their growing presence on the market. With the increasing share of products containing probiotics, the scientific research on the criteria for their selection and the mechanism of health-promoting effects, expands and deepens. Comparative studies on some probiotic properties of three strains of *Bifidobacterium longum* from human origin, were performed. The in-vitro survival of bifidobacteria in artificial gastric juice, tolerance to bile salts and the ability to reduce serum cholesterol were tested. The obtained results show, that strain *Bifidobacterium longum 1/2* has superior probiotic properties with respect to survival of the cells in artificial gastric juice with pH 3.0, bile salt tolerance with concentration 0.3% and ability for reduction of serum cholesterol within 30%.

The proven probiotic characteristics of strain *Bifidobacterium longum 1/2*, determine the strain as suitable for inclusion in new functional probiotic foods.

**Key words:** probiotics, *Bifidobacterium longum*, probiotic properties

## GAM50

# DEGRADATION OF MIXED AROMATIC POLLUTANTS BY TRAMETES VERSICOLOR STRAIN 1

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One of the most distributed groups of industrial pollutants consists of aromatic hydrocarbons. For the reason of the complex nature of such wastes the obtaining of new strains able to degrade different toxic aromatic compounds in mixtures is a challenge to many scientists exploring the area of environmental microbiology.

In this study the strain of white rot fungus *Trametes versicolor* was cultivated in a medium comprising a mixture of phenol (0.2 g/l), resorcinol (0.1 g/l), p-cresol (0.1 g/l), and o-nitrophenol (0.14 g/l). Phenol, resorcinol and p-cresol were degraded in 24 hours and than a slow o-nitrophenol degradation was observed. There was about 50% decrease of o-nitrophenol concentration in 144 hours.

The degradation of the tested concentrations of phenol, resorcinol and p-cresol in a mixture showed similar characteristics as in experiments carried out with each one of them used as a single carbon substrate. The data showed that the presence of o-nitrophenol does not influence the biodegradation rate of other compounds included in the mixture. The capability of the investigated strain obtained demonstrated its potential for future application in bioremediation technologies oriented to cleaning and preservation of industrially polluted water.

Key words: *Trametes versicolor*; aromatic pollutants biodegradation

## GAM51

# SEQUENCE ANALYSIS OF GENE CODING FOR PHENOL HYDROXYLASE IN *ASPERGILLUS AWAMORI* STRAIN

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Some members of Aspergillus genera are reported to exert a significant capacity in aromatic compounds catabolism. In the present study the panfungal primer pair was used in PCR procedure with the purpose to approve the taxonomic affiliation of a strain *Aspergillus awamori* NRRL 3112 by 18 S rDNA sequence analysis. The investigated strain is able to degrade various aromatic compounds.

The first key enzyme of aromatics degradation is phenol hydroxylase (EC 1.14.13.7) which catalyzes the conversion of phenols to their *o*-diol derivatives by incorporation of a single hydroxyl group into the substrate. The aim of the present work was to identify and sequenced the gene encoding the phenol hydroxylase in a strain of *Aspergillus awamori*. A three pairs of oligonucleotide primers were created on the bases of sequences for proteins with phenol hydroxylase activities registered in NCBI. A 2071 bp DNA sequence was obtained as a result of carried out PCR and sequence analyses. The established nucleotide and related protein sequences were analyzed and compared with that of other enzymes with similar properties. All sequences obtained in this investigation have been deposited in the NCBI nucleotide sequence databases under accession numbers GQ472777, GQ279378, and ACT52897.
Key words: *Aspergillus awamori*; phenol hydroxylase; PCR; sequence analyses

## GAM52

# DECOLORIZATION OF TEXTILE DYE REMAZOL BRILLIANT BLUE R BY MYCELIAL CULTURE OF WHITE-ROT FUNGI *TRAMETES VERSICOLOR* 1

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The industry is major source of pollution for water ecosystems. Production of textile, cellulose and some chemicals in factories is connected with the use of synthetic dyes witch are potential environmental hazard in discharged effluents. The biological treatment is a very perspective, environmentally protective and low cost approach for synthetic dyes removal. Remazol Brilliant Blue R is an anthraquinone-based dye widely used in the textile industry for coloring denim and other cotton ware.

Decolorization of this dye by *Trametes versicolor* 1 strain was investigated. The experiments for decolorization of 125mg/l Remazol Brilliant Blue R were carried out in the medium completed with different glucose concentrations (1, 2 and 3 %). The laccasse activity (EC 1.10.3.2) was measured during the studied process. It was shown that there is a direct correlation between the enzyme activity dynamics and the decolorization process effectiveness. The influence of glucose concentration on specific rate of decolorization was analyzed. Despite of the negative effect of higher glucose concentrations on the specific rate of decolorization the best conditions established for the total decolorization of 125mg/l Remazol Brilliant Blue R dye were found in the medium containing 3% glucose. The period of time for laccasse production by the cells was significantly increased in medium with higher glucose concentration. The maximal laccase enzyme activity observed was 65 U/ml at the 144$^{th}$ hour of the process and 100% dye decolorized was achieved in 384 hours.

Key words: Decolorization, Remazol Brilliant Blue R, *Trametes versicolor*

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**GAM53**

# REVERSIBILITY AND STABILITY OF SUPEROXIDE DISMUTASE, PRODUCED BY FUNGAL STRAIN *HUMICOLA LUTEA 103*

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The glycosylated SODs are of interest both from scientific and clinical point of view. Therefore the stability of naturally glycosylated Cu/Zn-superoxide dismutase (HLSOD), isolated from fungal strain *Humicola lutea* 103 was analyzed by circular dichroism at different pH and temperature. To provide information about the stability of the enzyme a pH–T diagram, typical phase portrait for stability and denaturation reversibility was obtained. By analyzing the *T* transition curves of HLSOD at different pH values, several parameters of the thermodynamic functions were obtained. The start of the acid and alkaline denaturation were calculated to be below pH 5.6 and above pH 9,5, respectively. Increasing the temperature from 20 to 53°C, the reversibility of the protein also increases, opening a reversibility window within the range of pH 4.0–9.5. On analyzing the termotransition curves no changes in the ellipticity were observed in the region between 20 and 45°C. For this range, the thermodynamic functions $\Delta H°$ and $\Delta G°$ for a standard temperature of 25 °C were calculated. Based on the primary structure the 3-D model of one subunit of the enzyme was billed. The position of the carbohydrate chain was identified to be on the surface of the molecule and probably it influence on the stability of the enzyme.

Overall model of HLSOD showing the secondary structure elements. The figure was drawn with MOLSCRIPT and Raster3D

# VIROLOGY

## V1

## *CHRISTO RUSSEFF* MEMORIAL LECTURE:

## BLUETONGUE DISEASE IN RUMINANTS – HISTORIC REVIEW OF THE DISEASE IN EUROPE – PERSPECTIVES AND PROBLEMS OF PROPHYLAXIS AND CONTROL

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Bluetongue Disease (BT) is a World Organization for Animal Health (OIE) reportable disease and is of considerable socio-economic concern and of major importance in the international trade of animals and animal products. Before 1998, BT was considered an *exotic disease* in Europe with just a few sporadic incursions (e.g., Spain and Portugal from 1956 through 1960)

BT is an infectious but noncontiguous viral disease caused by Bluetongue virus (BTV). The virus belongs to the family Reoviridae, genus Orbivirus; there are 24 serotypes. The viral genome consists of 10 double-stranded RNA segments that encode for 4 nonstructural proteins (NS1, NS2, NS3, and NS3A) and 7 structural (VP1-VP7) proteins. BTV serotypes 1, 2, 3, 4, 6, and 10 have a high pathogenic index and high epidemic potential. However, a high genetic diversity of BTV exists that is a consequence of both drift (i.e., point mutations) and shift (i.e., reassortment of individual BTV gene segments) so pathogenicity even within a serotype may be highly variable.

The aim of the article is to provide a synthesis of history of last epidemic wave in Europe 2006-2009 and some perspectives of BT epidemiology and control in the European Union (EU) since BTV's introduction in 1998 to the continent through the Balkan peninsula. For better understanding, we provide a short overview of the epidemiologic situation in Europe, followed by a brief description of the susceptible species, a discussion of the vectorial capacity and competence of the Culicoides spp. vectors, factors fascination the enormous spread of the disease trough the Western Europe, to outline of the modes of introduction, mechanisms of amplification and problems around its control and prophylaxis of BT.

**V2**

## *CHRISTO RUSSEFF* MEMORIAL LECTURE:

# AVIAN INFLUENZA IN BIRDS: REVIEW OF INFECTION

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Influenza A viruses of all known subtypes are maintained in the Orders Anseriformes (i.e., ducks, geese, and swan) and Charadriiformes (i.e., gulls, terns, surfbirds, and sandpipers). Based on their pathogenicity in chickens, avian influenza A viruses can be divided into viruses of high or low pathogenicity. All highly pathogenic avian influenza viruses known to date belong to the H5 or H7 subtype.

In examination were followed: history of HPAIV, Natural hosts in Wild Birds, Domestic hosts, Transmission Cycle **,** Simptoms, Ecology and Epidemiology of H5N1, Spread and Isolation of AIV in Bulgaria.

Conducted by our research included:

**1. Period of shedding of the avian Influenza A H6N2 subtype virus isolate in different domestic poults (ducklings, geeses, turkeys and chickens)**

Virus was re-isolatedfrom all the ducklings (100%), from 56% of the turkey poults and from 33%of the chickens essentially during the first 10 days after virus inoculation.Positive cloacal samples prevailed compared to the oropharyngeal samples(the percentages of positive cloacal samples among the overall positive sampleswere 72%, 83% and 80% in ducklings, turkey poults and chickens respectively).The mean virus shedding periods determined from cloacal samples were10.6, 6.6 and 4.3 days in ducklings, turkeys and chickens respectively andthe longer periods observed in this study were 21, 10 and 5 days respectivelywhereas the oropharyngeal virus shedding periods were shorter in the 3 species.Consequently, cloacal samples may be more relevant for exploring spontaneousavian influenza cases.

**2. Use of a commercial immunoassay for rapid detection of influenza A antigen in ducks**

The overall proportion of samples positive by virus isolation was 34·5 per cent (50 per cent of the cloacal samples and 19 per cent of the oropharyngeal samples). Virus ould be isolated from the cloacal from the oropharyngeal samples only until day 10 postinfection.The number of isolates varied with time.

Most were obtained early in the infection; on day 3 postinfection, five of seven cloacal samples and five of seven oropharyngeal samples (71·4 per cent) were positive, but on day 21 only two of seven (28·6 per cent) cloacal samples were positive.

**3. Influence of inoculation dose of avian H6N2 influenza A virus on virus shedding and humoral immune response of chickens after artificial experimental intravenous infection**

The virus reisolation showed that infection was established in chickens, infected with dose of $10^{5.25}$ ELD50 /100 μL per bird and $10^{4.25}$ ELD50 /100 μL per bird. More birds with virus reisolation were detected when inoculation was made with the higher virus dose (33.33% versus 16.67%), with higher number of isolates (6% versus 1.2%). Antibodies were detected in 100% of the birds, infected with virus dose of $10^{5.25}$ ELD50 /100 μL per bird and in 16.67% of the birds, infected with a dose of $10^{4.25}$ ELD50 /100 μL per bird. Antibody titers of birds with virus reisolation varied within 8 log2 – 11 log2, and those of the birds without virus shedding (only the ones, infected with a dose of $10^{5.25}$ ELD50 /100 μL per bird) – within 4 log2 - 5 log, with later appearance of antibodies (since day 14 PI).

**4. Survival of avian influenza viruses in filtered and natural surface waters of different physical and chemical parameters**

Avian influenza viruses (AIV) survival was studied in five basins of different physical and chemical parameters of water, including pH, temperature and salinity. Places are known for the considerable aggregation of wild waterfowl. After 1:1000 dilution H6N2 isolate with titer $10^{1.63}$ EID50/0.1 ml water and A/duck/ England/56 H11N6 with titer $10^{3.83}$ EID50/0.1 ml water were used. Survival did not exceed 24 h at 9.34 pH, reached one week at the highest salinity and more than 2 weeks at close to tap water physical and chemical characteristics. Presence of living microorganisms in some waters further reduced AIV survival (with 12.5 % in H6N2 and with 14.29 % in A/duck/England/56 H11N6 in the waters with microorganism count as small as $8{,}9.\ 10^{4}$ cells/ ml). When microorganism count was high ($6{,}64.10^{6}$ cells/ml and $5{,}2.10^{6}$ cells/ ml) the period of survival was with 40 % and 35.71 % shorter. Titer values in waters containing microorganisms were with 0.17 $\log_{10} EID_{50}$/0,1 ml to 2.16 $\log_{10} EID_{50}$/0,1 ml lower.

## V3

# EMERGING VIRUSES IN GREECE: CRIMEAN CONGO HEMORRHAGIC FEVER VIRUS IN 2008 – WEST NILE VIRUS IN 2010

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Greece is at the meeting point of three continents, Europe, Asia and Africa, and microorganisms, especially vector-borne RNA viruses can easily be transferred and established themselves in the country causing sporadic cases or even outbreaks. Two recent examples are Crimean-Congo hemorrhagic fever virus (CCHFV) and West Nile virus (WNV) which emerged in Greece in 2008 and in 2010 respectively, and caused one fatal human CCHF case and a large WNV outbreak.

CCHFV (genus Nairovirus, family *Bunyaviridae*) is transmitted to humans by tick bite or by direct contact with infected blood or tissues, causing sometimes family or nosocomial outbreaks. Main clinical symptoms are high fever and hemorrhagic manifestations. Although CCHF cases have been reported in Bulgaria, Kosovo and Albania, the disease has never been detected in Greece before 2008, when a 46-year-old woman with disseminated intravascular coagulation died after a tick bite in Komotini city, Northwestern Greece. An RT-nested PCR resulted positive, while a quantitative real time PCR revealed high CCHF viral load, as is usually seen in fatal cases. Sequence analysis showed that the causative CCHFV strain was similar to strains detected or isolated in the Balkan peninsula, Russia and Turkey. No other CCHF case was observed in the next years. However, seroprevalence studies showed that there are certain areas in Greece where human population has IgG antibodies against the virus.

WNV (genus Flavivirus, family *Flaviviridae*) is transmitted to humans by mosquito bites, while transmission by blood transfusion or organ transplantation is also possible. It causes to humans a febrile syndrome, while approximately 1% of the infected people present a neuroinvasive disease (mainly encephalitis). WNV was known for many years in Africa; the interest increased after the 1996 outbreak in Romania, the emergence of the disease in North America in 1999, and the outbreak in Italy in 2008 and 2009. A large WNV outbreak in currently ongoing in Central Macedonia, Greece, with more than 250 cases reported up to now, mostly among elderly persons with an underlying disease. The fatality rate among the neuroinvasive cases is approximately

12%. Most affected areas are close to rivers, lakes or canals, and it is suggested that the virus has been transferred by migratory birds. WNV lineage 2 was detected in a pool of *Culex pipiens* mosquitoes collected at the site where a clinical case was observed. At the same time horses with neurological symptoms have been detected, while dead birds have been also observed. Data suggest that WNV lineage 2 is at least as pathogenic as lineage 1 and that strains belonging to this lineage have been already established in Europe. Active surveillance is needed for the rapid recognition of the virus activity in spring and summer and guidelines have to be given to people to prevent mosquito bites, while control programs have to be implemented to eradicate mosquitoes.

## V4

## DIFFERENT MECHANISMS OF INTERACTIONS BETWEEN HIV AND VIRUSES CAUSING OPPOTUNUSTIC AND CO-INFECTIONS IN HIV INFECTED PATIENT – AN EVIDENT- BASED HYPOTHESIS

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**Background**

Viral opportunistic infections (OI) and co-infections are still frequent in HIV-infected patients despite their high reduction by HAART. **T**he aim of the present study was to provide evidence supporting a hypothesis about the different mechanisms of interaction between HIV and viruses causing opportunistic infections, from one side ( Progressive Multifocal Leukoencephalopathy - PML) (Human polyoma Virus BK, JC or both ) and co-infections (Flu) – from the other. Since HAART was introduced, the decrease in the incidence of the PML was not as frequent as other opportunistic infections. On the other hand, co-infection with FluB often occurs in people living with HIV. Clinically, it is represented by an increase in HIV viral load, often compromising the successful therapy .**Methods** Forty-nine Bulgarians with HIV, all treated by HAART, without neurological and/or kidney disorders, were tested for JCV and BKV and genotyped for CCR5 (CCR5del32), CCR2 (CCR2-64I), and

CXCR4 (SDF1-30A) by specific PCRs.

An experimental model of in vitro co-infection of HIV-1 + FluB virus was created. Further, sialylation patterns of HIV-1 chronically infected cells - controls or co-infected with Flu B virus were compared. H9/HTLVIIIB cells were infected with FluB/Brisbane/60/2008 and the FluB virus replication was confirmed by Real-time PCR. **Results** In 27/49 (55.1%) individuals a co-infection with HPoV was identified—BKV in 12/49 (24.5%), JCV—in another 12/49 (24.5%), and both viruses—in 3/49 (6.1%). A high frequency of wild type (wt) of CCR5, CXCR4 and CCR2 was found in patients with HPoV. The number of individuals bearing wt of all co-receptors in the group of persons not infected with HPoV was significantly lower ($P<0.03$) than that with polymorphism/s. in one or two genes (SDF1 and CCR2) in the same group. Here we report that the increase in HIV replication (viral load ) during HIV + Flu mixed infection happens after desialylation and temporarily decrease of HIV viral replication (measured by infectivity and RT) during the first 72-96 h after co-infection. **Conclusions** We found two different mechanisms of interaction between HIV and Human Polyoma Viruses and HIV and Flu virus B . When an opportunistic infection (such as HPoV) is being demonstrated , the importance of the host factors - namely coreceptors CCR5, CCR2, and CXCR4 is not only for HIV, but also for HPoV. Only functionally capable receptors coded by wild-type (wt) genotypes could facilitate internalization of HPoV in the cell resulting in brain and/or kidney infection/s in HIV infected individuals . As demonstrated , this happens even without expressed immunodeficiency , in the face of Highly Active Antiretroviral Therapy (HAART).

When co-infection with FluB virus occurs, the increase of viral replication (expressed clinically as increased viral load) happens after a kind of chemical interaction rather than as interaction with host genetic factors . Here a desialylation of gp120 of HIV by the Flu sialidase is demonstrated . A similar interaction - namely between GBV-C virus E2 envelope and HIV fusion protein gp41 is proposed as a mechanism potentially modifying its conformation and leading to a decrease of HIV replication in GBV-C virus co-infection . Additional studies of mixed infections of HIV with other viruses could confirm and enrich these findings.

**V5**

# DYNAMICS OF HIV-1 REPLICATION AND INFECTIVITY IN EXPERIMENTAL CO-INFECTION "HIV-1 + HUMAN INFLUENZA (FLU) VIRUS" – IMPLICATION TO PATHOGENESIS IN HIV-INFECTED INDIVIDUALS

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Earlier, we have reported a hypothesis that exogenous neuraminidase of Flu virus through desialylation of HIV-1gp120 changes its configuration, so becoming unrecognizable by epitope-specific antibodies. Here we try to see the initial changes leading to the well-known fact that after Flu co-infection the HIV viral load is clinically increasing even sometimes compromising Highly Active Antiretroviral Therapy (HAART).

• To study this, an experimental model of in vitro co-infection of HIV-1 + Flu virus should be created. Further, exploring the newly obtained model, sialylation patterns of HIV-1 chronically infected cells - controls or co-infected with Flu B virus were compared. H9/HTLVIIIB cells were infected with FluB/Brisbane/60/2008 with three different viral doses ($10^0$, $10^{-1}$ and $10^{-2}$), after UV inactivation of hemagglutinin. Co-infection with FluB and its replication were confirmed by Real-time PCR with appropriate primers provided by CDC, USA. Gp120 (pg/ml) (HIV-1 gp120 Antigen Capture Assay, Advanced BioScience, USA) and reverse transcriptase (RT) (pg/ml) (HS-kit LentiRT-Cavidi, Sweden) concentrations in H9/HTLVIIIB supernatants in both HIV controls and co-infected with FluB cells were measured and the results were averaged for each viral dose of FluB. Twenty four hours after co-infection cells were labeled with [14C]N-AcetylMannoseamine - a precursor of sialic acid biosynthesis. The sialoglycoprotein patterns were obtained by preparative isoelectrofocusing. Each fraction was measured for $^{14}C$-incorporation and

RT activity. A well expressed desialylation of gp120 after FluB treatment was observed in sialoglycoprotein patterns of co-infected with Flu cells compared to HIV-controls. Desialylation was confirmed by decrease of gp120 concentration. Simultaneously, RT after Flu virus co-infection also decreased. Probably this happens during the first hours of co-infection but after 96th hours the RT showed a trend to recover and even to outrun the values at 24$^{th}$ hour after co-infection. The increased RT activity together with higher infectivity and cytopathicity of HIV-1 after co-infection with FluB virus at $10^0$ and $10^{-1}$ represents a good explanation to the increase of viral titer. At 72-96$^{th}$ h after co-infection the titer of HIV-$1_{IIIB}$ control = 2 x $10^6$ TCID/ml and that of HIV-1 co-infected with flu B > 2 x $10^7$ TCID/ml – a fact well coinciding with the increase of viral load. Monoclonal antibodies (MAb) against HIV-1 gp120 (broadly reactive) and gp120 V3 (307-320 aa) (Advanced Bioscience,USA) were used in neutralization assays to study the changes in gp120 after FluB treatment. V3 MAb but not broadly reactive MAb against gp120 recognizes and neutralizes the newly formed by FluB neuraminidase epitopes on HIV-1 virion.

Co-infection with FluB often occurs in people living with HIV and here is presented an experimental model to study it. Clinically, it is represented by an increase in HIV viral load, but here we report that this happens after desialylation and decrease of HIV viral replication (measured by infectivity and RT) during the first 72-96 h after co-infection. After that a recovering and even more intensive replication and infectivity of HIV follows. The data fit well to glycan shield model saying that during desialylation the receptor/co-receptor binding is not impaired, so explaining HIV more active replication and infectivity observed in this study. Desialylation of HIV-1 gp120 directly affects HIV replication and infectivity and this mechanism of action of Flu virus is quite different from that linked simply to immune deficiency in HIV-infected people.

## V6

## APPROACHES FOR THE ENHANCEMENT OF THE ANTIENTEROVIRAL EFFECT OF OXOGLAUCINE

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Enteroviruses are known to cause a wide variety of syndromes and diseases ranging from asymptomatic or maild malaise to severe and even life-threaten-

ing conditions. This imposes the search for new inhibitors of enterovirus replication, as well as for ways to enhance the antiviral effect of the known ones.

Recently the broad spectrum antienteroviral effect of oxoglaucine (OG) has been described. OG successfully inhibits the replication *in vitro* of polioviruses, coxsackieviruses B, a series of echoviruses and human rhinovirus 14 revealing in many cases a selectivity index of 100 and even higher. But expectedly rather rapid development of resistant progeny has been observed.

Aiming further enhancement of the antiviral effect, OG was combined in dual concomitant combinations with several known picornavirus inhibitors, i.e. disoxaril, 2-(α-hydroxyibenzyl)benzimidazole (HBB), guanidine hydrochloride, 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile (DNB) and ribavirin. The effect of the combinations was studied as regards the replication of poliovirus 1, strain Lsc-2ab (representing human enterovirus C) and coxsackievirus B1 (representing human enterovirus B).

All combinations of OG were additive or synergistic ones with the exception of those with ribavirin. The latter were mildly antagonistic. The highest volume of synergy was observed when OG was combined with DNB. The synergistic and additive combined effect was better expressed on the replication of poliovirus 1.

In conclusion, to achieve the greatest benefit from the application of OG in terms of reduction of virus replication, decrease the toxicity and delay of resistance development, the compound should be combined in at least two-drug regimen.

## V7

## ANTI-HIV ACTIVITY OF A NEWLY SYNTHESIZED ESTER OF ABACAVIR–A-GLY

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Background: Abacavir is a synthetic carbocyclic guanosine analogue with inhibitory activity against HIV. Clinically, hypersensitivity reactions, cardiovascular risk, etc. have been associated with abacavir. A possible way to minimize these effects is by modifying the chemical structure of known antiviral drugs.

Aim: The aim of this study was to synthesize esters - prodrugs of abacavir using thiazole-containing amino acids (glycine - Gly) and to evaluate their activity on HIV replication in cell culture compared to abacavir (ABC).

Methods: ABC derivatives designated A-Gly-ABC and Gly-Gly-ABC were synthesized in Dept. of Chemistry, South-West University "Neofit Rilski", Blagoevgrad,, Bulgaria. Cytotoxicity and maximal non-toxic concentrations (MNC) were detected on MT-2 cells. The effect on HIV-replication was measured by infectivity (MTT assay) and by Reverse Transcriptase (RT) activity in supernatants of MT-2 cells infected with HIV-1 III B lab. strain. The impact on recombinant RT (rRT) was also measured. Additionally, mitochondrial toxicity was evaluated by RT-PCR as a ratio of mtDNA:nDNA. Quantification of mtDNA was achieved by amplification of a highly conserved region of the mtDNA-encoded subunit II of cytochrome c oxidase (COII) gene and nDNA – by amplifying the nuclear ASPG gene. Further, 32 passages of HIV-1 in MT-2 cells with increasing concentrations of A-Gly-ABC were carried out and the resulting virus (RT region of the genome) was sequenced using Opengene, TRUGENE test kit (Siemens).

Results: Gly-Gly-ABC was more cytotoxic than A-Gly-ABC which in turn proved less cytotoxic than ABC. Both A-Gly-ABC and ABC showed similar anti-HIV activity by infectivity and RT inhibition in supernatants. Both demonstrated no direct inhibition of rRT probably due to lack of cell phosphorylating enzymes while performing this test. IC50 of A-Gly was increased up to 8-9 fold compared to initial IC50. Mitochondrial toxicity was low and comparable to that of ABC. The other compound – Gly-Gly-ABC was not further evaluated due to its high cytotoxicity.

Conclusions: A new derivative – A-Gly-ABC was synthesized with anti-HIV activity comparable to ABC by inhibition of infectivity and RT. Currently, further passaging of HIV with increasing concentrations of A-Gly-ABC and looking for mutations is in progress.

**V8**

# TWO NEWLY SYNTHESIZED 2-STYRYL-8-HYDROXY-QUINOLINES (8-SQs) DERIVATIVES WITH ANTI-HIV-1 ACTIVITY TARGETING VIRAL INTEGRASE AND PROTEASE

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Background: Earlier, it has been reported by Bonnenfant ,S. et al. (2004) about anti-HIV-1integrase activity of styryl-quinolines. In Trakia University, Stara Zagora, Bulgaria 2-styryl-8-hydroxyquinolines (8-SQs) derivatives were synthesized and evaluated for anti-HIV-activity in cell culture (Hinkov,A. et al, 2010). Two of them – designated 105B and 241 (differing in substitutes at C2 in the phenol ring) demonstrated well-expressed anti-HIV-1 effect in MT-2 cells and were further studied to target this activity .

**Aim** of this work was to target anti-HIV-1 activity of 105B and 241 following the algorythm earlier described by us to study putative HIV inhibitors of cell culture.

Methods: As a source of HIV-1 supernatants of chronically infected H9/HTLVIIIB were used. Cytotoxicity tests (% cell survival) and microtiter infection assays based on HIV cytopathic effect in MT-2 cells were read by MTT uptake assay. Reverse transcriptase (RT activity) in supernatants and directly on recombinant RT (HS-Lenti RT Activity test, Cavidi, Sweden) were checked. Mitochondrial toxicity was evaluated by RT-PCR as a ratio of mtDNA:nDNA. Further, protease as a target was studied by modified screening method using direct specrtophotometric reading of specific substrate utilization by native HIV-1 protease in absence and presence of the substances studied. To check the integrase as a target, mutants were obtained by serial passging of virus

with continious exposure to increasing concentrations of active compounds followed by sequencing of IN region.

Results: Both 105B and 241 demonstrated inhibition of HIV replication by microtiter infectivity assay (105B > 95% and 241 ~ 70%.).105 B showed mitochondrial toxicity (ratio mtDNA:nDNA=0,82 compared to 0,97 and 1,01in MT-2 uninfected cells and HIV-1 infected MT-2 cells without inhibitor resp.) accompanied by reducing of both mitochondrial and nuclear DNA. 241 was not toxic to mitochondria. No RT inhibition was found. Also, both derivatives exposed anti-protease inhibition (105B – 21% and 241 – 25% resp.). As far as anti-protease activity did not explain anti-HIV effect in microtiter assay, we looked for mutations in IN gene of passages Nos 30 – 32 for 105B and 241. The molecular sequencing found mutations in IN region.

Conclusions: Two new 8-SQs were synthesized with anti-HIV activity. The chemical structure of integrase inhibitors (INIs) combined with their mechanism of action in HIV-infected cells shows two different groups of INIs: compounds inhibiting other (except integrase) viral targets and compounds with no other (except integrase) identified targets.Evidence demonstrate that 105 B and 241 influence more than one HIV-1 target – viral protease and integrase.

## V9

## PRESENCE OF RARE HIV-1 GENETIC FORMS: CRF 04_CPX, CRF 05_DF AND CRF 36_CPX IN BULGARIA

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**Background**

The aim of the present study was to determine HIV-1 genetic diversity among different risk groups in Bulgaria, by sequencing and phylogenetic characterization of 207 plasma samples from 1100 cumulative seropositive individuals diagnosed within 1986-2009. Few extremely rare HIV-1 genetic forms was discovered in Bulgaria, including CRF 05_DF, CRF 04_cpx and for the firs time CRF 36_cpx was found outside Africa.

**Methods**

We successfully performed HIV-1 genotyping on 207 out of 1100 cumulative seropositive individuals diagnosed within 1986-2009. Sequencing and genotyping of the Protease (PR) and Reverse Transcriptase (RT) of HIV-1 pol

gene was performed with Applied Biosystems Viroseq HIV-1 Genotyping System (Abbott Wiesbaden Germany) and/or TruGene DNA Sequencing System, OpenGene, Visible Genetics, Siemens. Bulgarian sequences, referent sequences from Los Alamos and the most similar GenBank sequences for each subtype and CRF were aligned using Bioedit. A maximum likelihood (ML) tree was then inferred with MEGA4.0 software. A probabilistic approach for detection of genomic recombinations in HIV-1 using jpHMM (jumping profile Hidden Markov Model) by jpHMM web server at GOBICS was performed.

**Results**

Of the 207 pol gene sequences, 153 were pure subtypes as follows: 104 (50,24%) subtype B, 24 (11,59%) subtype A1, 12 (5,80%) subtype C, 9 (4,35%) subtype F1, 4 (1,93%) subtype G and 2 (0,97%) subtype H. The phylogenetic analysis classified 54 sequences as CRFs and complex recombinant forms as follows: 32 (15,46%) CRF 01_AE, 14 (6,76%) CRF 02_AG, 4 (1,93%) CRF 05_DF, 1 (0,48%) 36_cpx and 1 (0,48%) was classified as 04_cpx.

**Conclusions**

We found high genetic diversity in Bulgaria, represented by 11 different genetic clades. Three of the genetic forms we found are extremely rare: CRF 04_cpx, 05_DF and 36_cpx. Various subtypes and CRFs have been introduced in the country emphasize the need for detailed surveillance and control of HIV-1 infection in Bulgaria.

## V10

## IDFLU: A NEW APPROACH TO THE SEASONAL ANTI-FLU VACCINATION

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**V11**

# АНТИОКСИДАНТИТЕ В ПРОФИЛАКТИКАТА И ТЕРАПИЯТА НА ГРИПНАТА ИНФЕКЦИЯ

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Грипната вирусна инфекция се съпътства от развитието на оксидативен стрес в организма, включващ активизиране на процесите на липидна пероксидация, понижаване съдържанието ендогенните нискомолекулни антиоксиданти, на чернодробния цитохром Р-450 и цитихром ц редуктазата, намаляване активностите на чернодробните лекарствометаболизиращи ензимни системи анилинхидроксилаза, етилморфин-N-деметилаза, амидопирин-N-деметилаза и аналгин-N-деметилаза. Приемът на антиксиданти като витамин Е, витамин С, флавоноидите кверцетин и рутин по профилактична схема преди вирусната инокулация показва протективен ефект по отношение на оксидативните увреждания и вирусната токсикоза. Нормализират се стойностите на биохимичните маркери на оксидативния стрес, както на 5-я, така и на 7-я ден след иницииране на инфекцията, причинена от грипен вирусен щам A/Aichi/2/68 (H3N2). Протективни ефекти по отношение на маркерите на оксидативните увреждания има и специфичните инхибитори на вирусната репликация римантадин и оселтамивир (тамифлу). Двата инхибитора имат различни механизми на действие, блокират различни етапи от вирусния репликативен цикъл. Приемът им по съответна терапевтична схема нормализира и стойностите на оксидативните маркери. Бихме могли да предположим, че римантадинът и оселтамивирът проявяват антиоксидантно действие. Нашите изследвания показват, че нито един от двата препарата не проявява антирадикалово действие по отношение на супероксидните, хидроксидните и хипохлоритните радикали, както и не въздейства върху индуцируемостта на процесите на липидна пероксидация. Римантадинът блокира М2 протеинът на грипните вириони, а тамифлу инхибира вирусната невраминидаза, което пречи на фирусната фузия в клетката-гостопиемник и понижава инфектируемостта, а от там и оксидативната патология в хода на грипната вирусна инфкция.

## V12

# EFFECT OF A NOVEL PROTEASE INHIBITOR FROM *STREPTOMYCES CHROMOFUSCUS* ON LUNG INJURY IN INFLUENZA VIRUS-INFECTED MICE AND ITS PHYSICO-CHEMICAL CHARACTERISTICS

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In the search of novel alternative approaches for the treatment of influenza virus infection we have found that the i*n vitro* anti-influenza virus effect of a novel proteinaceous protease inhibitor (SS 34-1), isolated from the culture supernatants of *Streptomyces chromopfuscus* was specific and selective. Further the protective effect of SS 34-1 in the experimental A/Aichi/2/68 (H3N2) influenza virus infection (IVI) *in vivo* was investigated. The SS 34-1-treatment of virus-infected mice (VIM) led to a significant reduction of mortality rate and marked prolongation of mean survival time. The lesions of the lungs due to infection as well as lung infectious virus titres were significantly reduced ($\Delta \log_{10} TCID_{50}/ml = 1.6 – 4.5$) in comparison to control VIM. The lung protease and the protease-inhibitory activities were followed for 9 days after infection. The application of SS 34-1 induced a continuous rise of the anti-protease activity but did not cause substantial changes in the lung protease activity of healthy mice. IVI triggered a slight reduction of protease activity in the lungs at 5 and 48 h p.i. and a marked increase at 24 h and 6 day p.i. Protease inhibition in the lungs was reduced at 24 and 48 h p.i. and an increase was observed at 5 h, 6 and 9 day p.i. SS 34-1-treatment brought both activities to normal levels.

The isolated novel protease inhibitor was purified by anion-exchange chromatography and reversed phase-HPLC analysis. It was a hydrophobic and a termostable protein, had a molecular mass of 11.2 kDa, isoelectric point of 7.5 and a high content of hydrophobic amino acids and proline. The N-terminal sequence demonstrated its homology to the *Streptomyces* subtilisin inhibitors family. Analysis by differential scanning calorimetry showed that at pH 7.5 the denaturation t° was 75 °C and the denaturation of PI was irreversible. Analysis by circular dichroism in the UV region 190-250 nm showed that at pH 7.5 the spectrum of the PI presented 2 minima at 208 and 222 nm, typical for an α-helical structure. At pH 2.5 and after heating the spectrum corresponded to an unordered structure.

**V13**

# PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF RESISTANCE TO NEURAMINIDASE INHIBITORS AND M2 BLOCKERS OF INFLUENZA SEASONAL STRAINS, ISOLATED IN BULGARIA 2004-2007

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The seasonal influenza A (H1N1 and H3N2) viruses are major causative agents of respiratory infections with a number of deaths every year.Two classes of drugs, M2 blockers (adamantanes) and neuraminidase inhibitors (NAIs) are currently approved for the prophylaxis and treatment of influenza A virus infections. Resistance acquired by viruses either in response to treatment or due to natural variation lessens the effectiveness of licensed antivirals. The frequency of antiviral drug resistance has increased dramatically over the last 10 years; reaching 100 % S31N H3N2 substituted mutants resistant to adamantanes in some countries At the beginning of 2007–2008 influenza season, a sharp increase of influenza A(H1N1) viruses with the H274Y oseltamivir resistance marker was detected in several countries in Europe and North America. Monitoring of antiviral resistance is therefore an essential component of influenza virus surveillance and therapy in Bulgaria and worldwide.

Molecular techniques and phenotypic tests were established for detection of resistance of influenza viruses H1N1 and H3N2 strains to oseltamivir and M2 blockers in clinical specimens, isolated in Bulgaria 2004-2007. $IC_{50}$ values of rimantadine were determined by reduction of CPE in MDCK cell cultures. $IC_{50}$ values of oseltamivir were evaluated by neuraminidase susceptibility assay with MUNANA fluorogenic substrate. RT-PCR and sequencing were carried out for evaluation of gene segments coding HA, NA and M2 proteins with subsequent phylogenetic analysis.

Overall 26 influenza strains H1N1 and H3N2, isolated in Bulgaria 2004-

2007 were assayed for their susceptibility to rimantadine in MDCK cell cultures - 22 were sensitive and 4 (two H1N1 and two H3N2) were resistant. 17 isolates were subjected to fluorescent assay and their susceptibility to oseltamivir and zanamivir were determined. $IC_{50}$ of zanamivir varied from 1.05 nM to 5.28nM and osletamivir $IC_{50}$ were from 0.28 nM to 1.31 nM. Further amplification and sequencing of M and NA and HA genes was performed for determination of mutaions associated with resistance to both groups of antivirals. In A/Sofia/1250 (H3N2) strain double two mutations in transmembrane region of M2 protein conferring resistance to adamantanes S31N and V27T were found. The virus was susceptible to neuraminidase inhibitors. In all other viruses no mutations associated with either to M2 blockers or neuraminidase inhibitors were determined.

The HA, NA, and M genes of the H1N1 and H3N2 viruses amplified with segment-specific primers DNA sequences were assembled, edited, translated and clustered and phylogenetic trees (dendrograms) were drawn. The phylogenetic trees of the genes were constructed using the neighbor-joining method and bootstrap analysis (n = 100). Bootstrap values P60% in the major branch were assessed as distinct clusters.

## V14

## SYNERGISTIC EFFECTS OF ELLAGITANNINS WITH ACYCLOVIR ON THE REPLICATION OF HERPES SIMPLEX VIRUS TYPE 2

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The effects of combinations of three nonahydroxyterphenoyl-containing C-glucosidic ellagitannins (castalagin, vescalagin and grandinin) with acyclovir (ACV) on the replication of sensitive and resistant to ACV herpes simplex virus type 2 strains in MDBK cell were tested according to the focus forming units (FFU, i.e. microscopically registered microplaques) reduction assay. The three-dimentional analytical approach of Prichard and Shipman was used to evaluate the character of drug-drug interaction. The 50% inhibitory concentration ($IC_{50}$) for each individual compound against HSV-2 was previously determined using FFU reduction assay and cytopathic effect (CPE) inhibition

test. The combination of ellagitannins and ACV demonstrated a marked synergistic effect on the replication of the BJA HSV-2 strain and PU HSV-2 ACV-resistant strain. Study on the combination effects of ellagitannins with ACV on the intact cell monolayers demonstrated a marked antagonism and lack of any traces of synergistic interactions.

## V15

## ANTIHERPES ACTIVIES OF SOME MEDICAL PLANTS FROM THE LAMIACEAE

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Chloroform, ethanol, methanol and water extracts, derived from wild and *in vitro* propagated *Lamium album L* and *Leonorus cardiaca* L. significantly blocked herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) replication in MDBK cells without apparent cytotoxicity. The chloroform extracts had most potent antiviral activity. The 50% effective doses ($IC_{50}$) of the chloroform extracts from native *L. cardiaca* were identically - 80μg/ml. The inhibitory effects of the other extracts were similar or slight.
The $IC_{50}$ value on the in vitro extracts from *L. album* was 550 μg/ml and 467μg/ml respectively and on the in vivo extracts were 668 μg/ml and 780 μg/ml. The viral replications ware suppressed with 90% after addition of the chloroform extracts in maximal nontoxic concentrations (MNC). The methanol *in vitro* extract and chloroform *in vivo* extract suppressed extracellular HSV-1 above 90% - Δlog4 and Δlog1.5 respectively.

**V16**

# APPLICATION OF REAL-TIME PCR IN COMPLEX DIAGNOSTICS OF INFECTIONS WITH EPSTEIN-BARR VIRUS

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Epstein-Barr virus (EBV) is a widespread human gamma-herpesvirus which is the etiological agent of infectious mononucleosis, as well as of broad spectrum of epithelio- and lymphoproliferative disorders.

The aims of this study were: 1/ to determine the diagnostic value of TaqMan real-time PCR assay in acute and reactivated EBV viral infections; 2/ to compare the EBV viral load in unfractioned blood and plasma; 3/ to determine the correlation between manual differential count, CBC, serologic responses and EBV viral load.

Whole blood, anticoagulated with K2 EDTA from healthy and suspective EBV diseased individuals was examined. DNA was extracted from unfractioned blood and separated plasma and the viral load was determined by TaqMan RT-PCR method using primer/probe set for BALF5 gene (viral DNA polymerase). Serologic markers EBV VCA IgM and EBV VCA IgG were evaluated by CLIA method and EBNA IgG by ELISA method.

The results showed sensitivity of TaqMan RT-PCR method about 1000 copies/ml (corresponding to 5 copies/PCR reaction) and linearity at least four orders of magnitute. In the clinical samples the assay detected EBV DNA in all patients with acute and in one with subacute EBV infection, as well as in two out of 10 healthy EBV carrier individuals. EBV DNA was found in blood from patients with acute infection before ascertainment of detectable positive values of EBV VCA Ig M . EBV viral loads in blood plasma was two orders of magnitude lower than tested in parallel whole blood.

Based on the obtained results we conclude:

1. TaqMan RT-PCR is sensitive and reliable method applicable for routine diagnosis and monitoring of EBV infections
2. Unfractined blood was assessed to be more appropriate biologic material for quantitative determination of EBV viral load in contrast to blood plasma
3. No correlation was found between the level of EBV viral load and the titre of IgG antibodies against EBV.

## V17

# NOVEL HUMAN POLYOMAVIRUSES – POSSIBLE CANCEROGENS?

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Polyomaviruses belong to a family of DNA tumorviruses that frequently cause cancer upon inoculation into heterologous hosts. Two members of the polyomavirus family, BK (HPyV-1) and JC (HPyV-2) were identified as human pathogens more than 40 years ago. Both are oncogenic when inoculated into newborn rodents. Their possible role for development of human cancers has been intensively investigated; conclusive results, however, are still missing. During the past years 5 new members of the polyomavirus family have been identified in humans: KI (HPyV-3), WU (HPyV-4), MCC (HPyV-5), (HPyV-6) and (HPyV-7). Whereas the first 2 were only found in respiratory fluids of children with respiratory infections and in healthy individuals, the MCC was found to be specifically linked to Merkel cell tumors, a rare human cancer of neuroendocrine origin. Latest analysis by Rolling Circle Amplification identified another two previously unknown polyomaviruses, named HPyV-6 and HPyV-7. The experiments show that their viral DNAs are shed from the skin in the form of assembled virions. Could there be new viruses that cause skin carcinomas?

## V18

# DIAGNOSTIC DIFFICULTIES WHEN STUDYING JC POLYOMAVIRUS IN TUMOUR SAMPLES

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Background: Polyomavirus JC (JCV) is an oncogenic virus associated with human tumours. There is growing evidence that JCV would initiate or promote cellular transformation, but the studies are inconclusive. The potential role of the virus to induce malignant transformation is related to integration in the human genome or to a “hit-and-run” mechanism which additionally complicates detection.

**Aim:** The current report discusses the diagnostic difficulties concerning detection of JCV and the role of the virus in the process of malignant transformation.

Methods: Different sensitive variants of the real-time PCR (SYBR Green, Taqman and LUX systems), were used, together with primers targeted at various segments of the viral genome.

Results: Patients with primary brain tumours (n=92) and cancer and precancer formations of the colon (n=44) were tested. Prevalence of the viral sequences varied from 20 to 49% among the different groups. A key factor was the use of fresh clinical samples. Investigations that failed to detect viral DNA worked with paraffin embedded tissues. JCV detection could be improved even more if amplification is performed after targeted laser capture microdissection. Another factor that might improve the results from the PCR amplification is pre-treatment with the enzyme Topoisomerase and the use of reagents that enhance specific amplification.

Conclusions: Improved detection of viral DNA is needed to clarify the role of JCV in the process of malignant transformation. The use of relevant clinical materials, sensitive methodology and novel reagents should be considered.

## V19

## DETECTION AND GENOTYPING OF HUMAN PAPILLOMAVIRUSES IN BULGARIAN PATIENTS FOR THE PERIOD OF 2009-2010

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Human papillomavirus (HPV) is now considered as a causative agent of carcinoma of the uterine cancer. Genital human papillomaviruses are classified into low-risk and high-risk. The low-risk HPVs are associated with genital warts and lesions, as for the high-risk they are commonly associated with high-grade squamous intraepithelial lesions and cervical cancer. Cervical cancer is the second most frequent cause of death from cancer in women worldwide; however cervical screening programs reduce the incidence of this type of can-

cer. Currently, two main approaches are used to screen clinical specimens for the presence of HPV – PCR with consensus primers that amplify a region of the major viral capsid L1 gene that is highly conserved among anogenital HPVs and DNA hybridization which detects the presence of either low-risk or high-risk HPVs, by formation of DNA-DNA hybrids. In this research we used Roche's LINEAR ARRAY HPV Genotyping Test. This method combines multiplex PCR with 40 pairs of primers and DNA hybridization. The products from the PCR are used to hybridize with nylon strips which containing immobilized DNAs of 38 types and subtypes of HPV genotypes. The cervical smears were collected from Bulgarian patients with condylata acuminate lesions, in three hospitals – Gynecology and Obstetrics "Majchin Dom", Sofia, "Tokuda Hospital", Sofia and from "National Institute of Oncology and Hematology", Sofia. One hundred and twenty six cervicovaginal smears and scrapings were tested by this method. Forty two percent of them were found positive for HPV DNA and fifty eight percent – negative. We found in twenty two samples HPV 16 DNA (38%), in eight samples - HPV 18 DNA (18%), in five cases - HPV 6 DNA (10%), in seven cases - HPV 53 DNA (11%), in three case – HPV 31 DNA (4%), in four cases – HPV 35 (5%) and in two cases we found positive for viral DNA types HPV 61 and HPV 62. Further studies are needed to determine the influence these HPV infections have on the epidemiology of the genital tract of these patients.

## V20

## GENETIC DIVERSITY OF HEPATITIS C VIRUS AMONG BULGARIAN INJECTING DRUG USERS WITH HEPATITIS C

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**Objective:** To assess the genotype diversity of hepatitis C virus (HCV) among bulgarian injecting drug users with hepatitis C.

**Methods:** Serum samples from 147 anti-HCV positive injecting drug users were tested by qualitative RT-PCR assay AMPLICOR Hepatitis C virus (HCV) Test, version 2.0 (Roche Molecular Systems, Inc, Branchburg, MJ, USA). Commercially available enzyme immunoassay ETI–AB-HCVK-4 (Dia Sorin, S.p.A. Italy) was used to detect anti-HCV antibody. Genotyping of HCV

RNA obtained from serum samples was performed using Versant HCV Genotype Assay (LiPa) – Bayer HealthCare LLC, Belgium.

**Results:** Since January till November 2008 a total of 147 anti-HCV positive serum samples from Bulgarian injecting drug users, were tested by RT-PCR for the presence of HCV RNA. One hundred and fifteen (78.2%) from the anti-HCV positive samples were positive for HCV RNA. The genotype was determined in 113 samples and 2 sera positive for HCV RNA couldn't be genotyped.

Genotype 1a was detected in 2 (1.7%) and genotype 1b in 72 (63.7%) of the samples. In only two cases with samples from genotype 1, the subgenotype couldn't be determined. Genotype 3a was present in 37 (32.7%) of the samples. No other genotypes were available in tested samples.

**Conclusions:** These results demonstrate that the genotype 1b of HCV is the most prevalent in Bulgarian drug users, but 3a genotype can be often detected also. These two genotypes (1b, 3a) of HCV are predominant in this high risk group.

## V21

## PARENTERALLY TRANSMITTED HEPATITIS VIRUSES IN CLINICALLY DIAGNOSED CASES OF ACUTE HEPATITIS AND CHRONIC LIVED DISEASES

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Hepatitis B virus (HBV) is the most important causative agent of blood borne hepatitis in humans. Hepatitis D virus (HDV) infection occurs either as a coinfection or superinfection in HBV positive persons. Hepatitis C virus (HCV) is the major cause of transfusion non-A, non-B hepatitis and continues to be a major cause of human liver disease throughout the world. **The aim** of the present study is to determine the relative deal of parentally transmitted hepatitis viruses in clinically diagnosed cases of acute hepatitis and chronic liver disease patients in North Eastern Bulgaria during the period 2002 – 2009.

**Material and methods:** A total of 2250 clinically diagnosed acute hepatitis patients and 1648 chronic liver disease patients were investigated. The serum samples were tested for HbsAg, IgM anti Hbc, HbeAg, anti Hbe, anti HCV

and anti HDV, using separate ELISA kits. **Results:** Of the 2250 acute hepatitis patients during the period 2004 – 2009 investigated, the relative deal of HBV is 14.66%, and of HCV – 3.28%. Coinfection or superinfection with HDV was confirmed in 7.57% and with HCV – in 4.8% of the HBV positive cases. Triple infections were proved in 1.8% of the cases. Of the 1648 chronic liver disease patients, HBV positive were 26.57%; 10.7% of them were coinfected with HDV. Anti HCV was detected in 13.8% of the patients. Fifteen (3.4%) of the HBV positive patients were simultaneously HBV and HCV positive. Four (0.91%) of the HBV positive patients were positive to HBV, HCV and HDV.

**Conclusions:** HBV is more common causative agent of acute viral hepatitis than HCV ($p<0.01$). HBV infection is the main cause of chronic liver diseases in North- Eastern Bulgaria. Screening of the clinically diagnosed patients for all viral causes is essential for establishing diagnosis and permit detection of the coinfected and superinfected.

## V22

## STUDY OF DIAGNOSTIC VALUE OF RNA-BASED HPV TEST NucliSENS EasyQ$^{tm}$ HPV

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The large dissemination of HPV infections makes the virus detection not enough effective way for identification of women with risk of cervical cancer (CC). DNA-based methods for determination of HPV show only the virus presence, but not its oncogenic activity. Newer technologies, like NASBA methodology (**N**ucleic **A**cid **S**equence-**B**ased **A**mplification), offer a new conception focused on more specific risk factors like the E6 and E7 oncogenes of the high risk HPVs. The mechanism by which HPV takes part in development of CC is connected with the expression of viral oncogenes E6 and E7, which by disturbance of the cell cycle control initiate the onset of cervical ongogenesis.

The aim of the study is investigation of the diagnostic value and establishment in routine praxis of RNA-based HPV test (NucliSENS EasyQ$^{tm}$ HPV), which utilizes NASBA technology for determination the expression of E6/E7 of five high-risk HPV types (16, 18, 31, 33 и 45).

Cervical cytological specimens taken with transport medium Thin Prep

from 200 clinically healthy and suspected ill individuals were analyzed. PCR DNA typing for 12 high-risk HPV ( 16, 18, 31,33, 35, 39, 45, 52, 56, 58, 59, 66) was performed with diagnostic kit HPV High Risk typing (Sacace Biotechnologies). RNA typing for E6/E7 oncogenes of five high-risk HPV (16, 18, 31, 33 и 45) was performed in parallel with diagnostic kit NucliSens HPV v1.0 (bioMerieux). All patients was made conventional PAP test, colposcopy with target biopsy and histological examination of biopsy.

It was found full correlation between the detected by PCR high-risk type HPV and the reported with NucliSENS EasyQ$^{tm}$ test presence of E6/E7 mRNA transcripts from the same HPV genotype, which confirms the high sensitivity and specificity of NASBA technology. This allowed women with early precancerous conditions of the cervix to be diagnosed.

Based on the obtained results we conclude that RNA-based NucliSENS EasyQ$^{tm}$ HPV is better prognostic factor for estimation of the risk for development of cervical cancer compared to PCR DNA detection.

## V23

## FLUORESCENCE ANTIBODY LABELING APPLIED TO CELLS INFECTED WITH VIRUSES AND BACTERIA

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Fluorescent microscopy allows us to visualize morphology of cell organelles and characterize membrane dynamics throughout the cytoplasm. Using antibodies to recognize membrane adaptor, traffic regulators and other specific factors would provide us with details about membrane composition and reorganization. This study is focused on one of the microtubule motor protein families, cytoplasmic dynein. Dynein is a minus end-directed motor along microtubule filaments involved in movements of the main organelles inside the cell. We are particularly interested in dynein heavy chain as well as in a key player from membrane transport in the early secretory pathway. Our first step is to characterize antibodies to these factors of protein transport and to use them further to describe if any effects are distinguished on membrane movements and Golgi/ERGIC morphology in infected tissue culture cells. This research provides use of prospective method to apply in investigation of viral infections and other pathogen causative agents.

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## V24

# ANTIOXIDANT PREVENTION AS A FACTOR REDUCING STRESS ULCERS IN ACUTE RADIATION SYNDROME

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OBJECTIVE: To follow the post irradiation recovery and antioxidant prevention with Respistim plus in experimental animals.

MATERIALS AND METHODS: Radiation injury is modeled with lethal dose ionizing irradiation on experimental animals (white male mice C3H with initial weight 23 gr.), divided in groups and exposed to radiation from $^{137}Cs$ with power 1,47 Gy/min. Respistim plus is administrated to those experimental animals *per os* for 15 consecutive days before irradiation. We study the following indexes: survival rate, levels of lipid peroxidation and stomach stress ulcers.

RESULTS: The results of our show that the radiation injury cause increase the levels of products of lipid peroxidation as well as increase the ulcer index in stomach and decrease the survival rate. Supplementation of mice with Respistim plus before irradiation leads to stomach protection against oxidative stress, increase the survival rate and decrease the value of the ulcer index in experimental group vs controls.

CONCLUSION: The obtained results give an opportunity to perform comparative analysis of survival rate, levels of lipid peroxidation (in stomach) and index of the ulceration. Respistim plus has protective effect on the survival rate in experimental animals, irradiated with ionizing irradiation. We suppose that the administration of respistim plus eventually have a positive effect on radiation casualties over post irradiation recovery.

# MEDICAL MICROBIOLOGY

## MM1

## THE COMMON DISEASE OF THE NAILS ONYCHOMYCOSIS

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**Introduction.** About a half of all nail abnormalities belong to onychomycoses. Fungal nail disease or onychomycosis is a common chronic infection. Onychomycosis can be caused by a number of different fungi (moulds and yeasts). The prevalence of onychomycosis in the adult population is between 2 – 13%.

**Objective.** Presentation of the spectrum of fungi causing onychomycosis in the past five years in our clinic in male and female persons.

**Material and methods.** The paper presents the results of mycological examinations of 443 materials sampled from various sites of nails in patients suspected of onychomicosis in the period between January 1, 2003. and December 31, 2007. Toenails and fingernails scrapings were sampled from lesions to sterile Petri dishes. Microscopic preparations clarified with 20% KOH were made from scrapings and searched for fungal elements. Each of the scrapings was cultured on Sabouraud medium with chloramphenicol and cycloheximid (bio Merieux). The culture was incubated at 27o and observed for four weeks. The fungi were identified on the basis of culture morphology, microscopic examinations and micro culture.

**Results.** Among 443 examined materials a number of 234 (52,82%) were positive. A number of 149 (33,63%) in females and 85 (19,19%) in males have had the positive result in scrapings of fingernails and toenails. The positive finding of fingernails in females were 50 (21,31%) and only 10 (4,27%) in males. The positive finding of toenails in females were 99 (42,31%) and 75 (32,05%) in males. The most frequent causative agent of onychomycosis of fingernails was yeasts of the genus Candida and of toenails was mould Trichophyton rubrum.

**Conclusion.** Onychomycosis of fingernails and toenails in females is more frequent in females than in males. The yeasts of the genus Candida mainly cause onychomycosis of fingernails in females whose hands are often submerged in water. The most common causative agent of toenails was dermatophytes.

**MM2**

# EVALUATION OF *CAGE* AND *CAGA* GENOTYPES IN *HELICOCACTER PYLORI* STRAINS FROM BULGARIAN SYMPTOMATIC PATIENTS

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The *cag* pathogenicity island (*cag*PAI), involving *cagA* and *cagE* genes of *H. pylori,* is an important virulence factor. In total, 219 patients with single strain infection were involved in the present study. Overall prevalence of *cagA*$^+$ and *cagE*$^+$ genotypes was 84.9% and 68.5%, respectively. Ulcer patients exhibited higher prevalence of strains with *cagA*$^+$ (93.8%), *cagE*$^+$ (84.4%), and *cagA*$^+$/*cagE*$^+$ (84.4%) genotypes than the rest (81.3%, 61.9% and 61.3%, respectively). The *cagE* gene, alone or in combinations, was associated with the age (>65 years) and sex (men). Virulent *cagE*$^+$ and *cagA*$^+$/*cagE*$^+$ genotypes, showing the strongest ($p \leq 0.001$) association with the diseases, are good markers for strain virulence and the prognosis of the infection. Testing of both *cag* genes is recommended to detect better the virulent strains. The results of the first Bulgarian study on *cag* genotypes in *H. pylori* suggests that *cagE* gene is an important virulence factor, which is associated, alone or in combination, with *cagA*$^+$ genotype and patients' characteristics, and should be widely involved in the studies on the prevalence of virulent *H. pylori* strains, which imply an aggressive eradication therapy.

## MM3

# ASSOCIATION OF *VACA* I ALLELES IN *HELICOBACTER PYLORY* WITH THE DISEASE, PLACE OF RESIDENCE AND PREVIOUS TREATMENT OF THE PATIENTS

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*H. pylori vacA* i1 allele in the intermediate region of *vacA* gene is a newly recognized virulence factor. Aim of the present study was to evaluate the prevalence of *vacA* i alleles of 216 *H. pylori* strains isolated from patients with gastroduodenal diseases. The prevalence of *vacA* genotypes was s1a 85.2%, s1b 1.4%, s2 13.4%, i1 63.4%, i2 36.6%, m1 40.7% and m2 59.3%. Ulcer patients harbored more often strains of *vacA* i1 and s1a/i1 genotypes than the reminder. Differences in the prevalence of *vacA* alleles between treated and untreated patients were found, moreover, a difference in *vacA* i1 prevalence between the groups according to the places of residence was detected as well. Unlike *vacA* s1 and s2, the prevalence of *vacA* i alleles was associated with the severity of diseases. The presence of *vacA* i types of low virulence can be associated with unsuccessful eradication of the infection; therefore a stringent control on the patients' compliance, especially in this group, can be recommended.

## MM4

# MODERN DIAGNOSTIC OF TICK-BORNE AND SOME ZOONOTIC INFECTIONS IN BULGARIA

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Modern diagnostic of Lyme borreliosis is based on detection of specific antibodies by ELISA with recombinant antigens, followed by confirmation of the positive results by immunoblot. Recently, peptide ELISA is introduced based on C6 region of VlsE antigen of *Borrelia garinii*, which is more sensitive and specific than any other ELISA and can replace the expensive immunoblot. Polymerase chain reactions (PCRs) based on detection of specific sequences of *fla*, *ospA* and 16S rRNA of *Borrelia burgdorferi* are developed and applied

to detect borreliae in skin and blood of patients, as well as to conduct studies in rodents and ticks. Currently introduced ELISA for diagnostic of another tick-borne infection in Bulgaria, Congo-Crimean hemorrhagic fever (CCHF), is much more sensitive than routinely used Complement fixation assay. Nested RT-PCR and RealTime RT-PCR based on detection of specific sequences in S segment of the viral RNA, allow detection of the CCHF virus in the first few days after the disease onset. Introducing ELISA and immunoblot in diagnostic of hantavirus infections allows not only to detect the virus but also to type it as Dobrava, Puumala and Saaremaa. In addition, RT-PCR followed by sequencing improved diagnostic capabilities and knowledge of which hantavirus types circulate in our country.

## MM5

## MINI-SYMPOSIUM OF AA MEDICAL BULGARIA OFFICIAL REPRESENTATIVE OF BIO-RAD ALTERNATIVE METHODS IN FOOD TESTING

Alexandra Livescu, Product Manager Emerging Markets, Bio-Rad

## MM6

## BIO-RAD SOLUTION FOR CLINICAL MICROBIOLOGY LABORATORIES

Florina Toma, BVD and CMD Product Specialist, Bio-Rad

## MM7

## BIO-RAD SOLUTIONS FOR WATER TESTING – CHECK 'N' SAFE YOUR BATHING WATER

Alexandra Livescu, Product Manager Emerging Markets, Bio-Rad

## MM8

## GlaxoSmithCline - SYMPOSIUM
## EUROPEAN VIEW ON VACCINE PROPHYLAXIS

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## MM9

## BIOLOGY AND UNIQUE MECHANISMS OF BEHAVIOUR *IN VIVO* AND *IN VITRO* OF MYCOBACTERIA L-FORMS AND TEIR ROLE IN LATENT TUBERCULOSIS

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Bacterial L-form conversion, i.e. existence without rigid walls, is universal but difficultly recognized phenomenon in nature. Experimental data indicate that L-forms of M. tuberculosis demonstrate atypical behavior and life style *in vivo*. They exist as unusual morphological units, such as large and elementary bodies, filaments, filterable granules, empty bodies and vesicles, which often remain stealthy and unidentified. Electron-microscopic observations show long-lasting persistence of L-forms outside or inside the phagocytes due to the incomplete and ineffectual phagocytosis. Occurrence and existence of L-forms *in vivo* suggest one of the possible pathways by which pathogens can survive and replicate within hosts for a long period may be associated with pathogenesis of latent tuberculosis.

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## MM10

# ANTIBIOTIC RESISTANCE CHARACTERISTIC AND STRAIN DIVERSITY IN *ACINETOBACTER BAUMANII*

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**Objectives:** *A. baumanii* is one of the major pathogen, causing healthcare associated infections.Since 2002, carbapenem resistant *A. baumannii* has emerged in Bulgarian hospitals. The aim of this study was to assess type diversity and antibiotic resistance including carbapenem resistance in multidrug-resistant *A. baumannii* (MDRAB) isolates from nine Bulgarian hospitals obtained between 2004 and 2008.

**Methods:** Investigated were 106 non-replicate MDRAB isolates including 58 prospective isolates from the Military Medical Academy (MMA) (2004-2006), 15 from from 3 other Bulgarian hospitals from 2005-2006, and 35 from 5 hospitals from 2008. Antibiotic susceptibility was determined with VITEK-2 . The organisms were identified to species (similarity cut-off level ≥50%), to clone (≥80%) and to strain (≥90%) by AFLP analysis. OXA-genes were detected by multiplex PCR.

**Results:** At the strain level (≥90%), isolates were allocated to 10 clusters (i.e. types) and 2 single strains. Predominant types were no. 3 (47 isolates), 8 (13 isolates), 10 (12 isolates)

and 12 (11 isolates). Isolates from the MMA comprised two major types, no. 3 (34 isolates) and 12 (11 isolates). Although most hospitals had a particular predominant type, several types were observed in multiple hospitals. With very few exceptions, types from 2004-2006 were distinct from those of 2008. Some clusters with isolates from multiple hospitals linked at ~80%, indicating clonal lineages. One of these, comprising types 5-10, was identified to EU clone I. Isolates were resistant to 8-15 out of 18 or 13-20 out of 21 antibiotics tested. Ninety-two isolates were carbapenem resistant. All isolates had a positive OXA-51 PCR, 81 were OXA-23 positive and 5 (in type 3 isolates from 2006) OXA-58 positive.

**Conclusion:** Results indicated a wide endemic or epidemic presence of different strains in the hospitals. Furthermore, there were indications for inter-hospital spread. The high degree of resistance to antibiotics including carbapenems is worrying.

## MM11

# MICROBIOLOGICAL CONTROL OF NOSE, MOUTH AND THROAT SWABS AS A MEASURE OF OBLIGATORY HEALTH SUPERVISION

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The aim of the paper is to show the dynamics of the swabs taken from the mouth, nose and throat of healthy staff in the Clinical Hospital- Bitola, as a measure of obligatory medical supervision. Material and method of work. The informations are obtained from the Center for Public Health-Bitola, for the period of 2005-2009. It is used a descriptive method of work, and the monthly and annual reports are used from the microbiological laboratory. Results. In the period of 5 years, out of 69,726 tested swabs from the mouth, nose and throat, 4964 (7.1%) are with microorganisms isolation.With swabs tested the common microorganisms are Staphylococcus aureus in 2910 samples (58.6%). Also β hemolytic streptococci are isolated in 506 (10.2%), Pneumococci are isolated in 819 samples (16.5%), gram positive cocci 124 (2.5%) and other gramnegative cocci 436 (8.8% ), and in 2021, 13 positive findings are isolated of Salmonella typhi. Conclusion. The health surveillance shoud be done regularly, it means taking swabs from the nose, mouth and throat of all health staff, attending to aseptic work conditions, wearing personal protection.

## MM12

# CONTROLLING INANIMATE ENVIRONMENT AS A MEASURE FOR ENFORCING THE HEALTH SURVEILLANCE LAW

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**The goal of this paper** is to show the existence of microorganisms in hospital environments, i.e. to foresee the efficacy of measurement enforcement of the health surveillance law.

**Materials and methods.** The data was taken from Public Health Center – Bitola and it covers the period of 2005-2009. Using data from monthly and yearly reports from microbiological laboratory, a descriptive analysis was made. The data implies to samples taken from sanitary and surgical materials

for sterility, by objects for general usage and sterilization control at the Clinical Hospital – Bitola.

**Results.** In the period previously mentioned, microorganism isolates have 49 (3.6%) of the total 1368 examined samples of sanitary and surgical material for sterility. Out of 3421 total examined samples of general use objects, pathogen isolates have 549 (16%), the sterilization controls didn't fit in 8 (1.2%) of the total 683 samples and, from the total 173 taken samples for pureness, 31 (17.9%) have positive findings.

**Conclusion.** Based on the given results, we were able to ascertain that continuing enforcement of the hygienic and technical measurements in hospital establishments, regular disinfection of inanimate surfaces and also toughening up of the enforcement of health surveillance is needed.

## MM13

## EFFECTIVE REAL TIME PCR SYBR GREEN SYSTEM FOR EARLY AND RAPID DETECTION OF *COXIELLA BURNETII*

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*Coxiella burnetii* is the cause for Q- fever that is spread all over the world and affects both, animals and humans. Q fever is a disease of great significance which may take a chronic or even lethal course. *Coxiella burnetii* is a potential biological agent that is possible to be used as a biological weapon. Prompt and specific diagnostic tools as well as elaborating new detection methods are needed for an exact diagnosis and a correct etiological treatment. One of the most contemporary monocular – biological methods for a prompt and specific detection at genetic level is the real-time PCR with conventional primers CB1/ CB2 and SYBR green dye.

We successfully tested experimental its specificity, sensitivity and effectiveness in sequence detecting of *Coxiella burnetii* genome which is strongly peculiar to that kind of microorganism.

**MM14**

# MODERN APPROACHES TO A RAPID DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* STRAINS IN BULGARIA

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The development in molecular epidemiological tools in the last 10 years had done much to unlock the secrets of the population genetics of tuberculosis by providing a reliable way to identify different strains of tuberculosis. Using spoligo-, IS6110-RFLP and 24-loci MIRU-VNTR typing methods it has been possible to plot the transmission between hosts over shorter and longer time periods. Multidrug-resistant tuberculosis (MDR-TB) in Bulgaria has reached alarming proportions (WHO estimation: 10.7% of new TB cases), much higher than in the EU neighbors. A correct and rapid detection of resistant strains is necessary for the appropriate and timely anti-TB therapy and reduction of total treatment cost. The prediction of drug resistance of *M. tuberculosis* by molecular tools presents a rapid alternative to the slow microbiological susceptibility tests. Characterization of molecular basis of drug-resistance in a survey area is a first step prior to development and implementation of such molecular-genetic methods. The obtained information will be used for timely prevention of TB via early diagnosis, adequate prevention and treatment of TB patients as well as for updating global genotype database and surveillance of dissemination of drug resistant TB in the world.

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## MM15

# SOME ASPECTS IN VIRULENCE OF *ECHERICHIA COLI*, ASSOCIATED WITH URINARY TRACT INFECTIONS

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We investigated antibiotic susceptibility and resistance to normal human serum of 35 strains *Escherichia coli* associated with different type urinary tract infections (UTI). Influence of different carbon sources and oxygen on serum resistance of strains was estimated. The presence of glucose repressed serum resistance of investigated strains either in presence or absence of oxygen. Among estimated strains nearly half (46%) show multidrug resistance phenotype. All investigated strains were susceptible to nitrofurantoin which often is drug of choice in hospital treatment of UTI.

## MM16

# FUNGICIDAL ACTIVITY OF HYBRID MATERIALS BASED ON POLYVINYLPYRROLIDONE WITH EMBEDDED SILVER NANOPARTICLES

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**Introduction:** The limited number of antimycotics in the medical practice and the presence of resistance towards part of them in clinical isolates raise the question to investigate the fungicidal properties of alternative means for prevention and treatment of diseases caused by fungi. Polyvinylpyrrolidone (PVP) called povidon is a water soluble polymer which possesses excellent wetting properties in aqueous solution and easily forms films. It is widely used in medicine, dentistry and pharmacy. The silver nanoparticles embedded in PVP are with proven antibacterial properties.

**Aim:** To test the fungicidal properties of the synthesized hybrid materials based on PVP with embedded silver nanoparticles, to establish the their mini-

mal fungicidal concentration (MFC) and standardized the test methods.

**Materials and methods:** The synthesis of the hybrid materials is described in [1]. Because of the lack of procedure for testing the fungicidal properties of the polymer with embedded silver nanoparticles, the test is performed by using a methodology which compiles the experience collected in approved standards of CLSI as M27-A2 [2], M-38-P [3]. Following the advices to the authors of articles in 2010 AAS [4] in bactericidal tests [5] additional tests are performed aiming conformation of the determined MFC.

**Conclusion:** The fungicidal properties of the synthesized hybrid materials with different silver concentrations are experimentally proven. The MFC for the yeasts and moulds after 48 hours exposition of the hybrid material with the suspension of the fungi is established. The established value is 2.4 ± 2.4 mg/ml. In the second method [5] 0.01 ml are inoculated after 24 hours exposition and the MFC for yeasts and moulds are specified. The MFC for C.albicans ATCC 10231 and A.brasiliensis ATCC 16404 is 4.9 mg/ml. The MFC for C.glabrata ATCC 90050 and C.tropicalis B 6030413 is 2.4 mg/ml, and for C.krusei ATCC 6258, the MFC is significantly lower – 0.15 mg/ml.

**References**: [1]Stanislav Nikolov, Rayna Bryaskova, Daniela Pencheva , Antibacterial hybrid materials based on polyvinilpyrrolidone with embedded silver nanoparticles, VII naucha posterna sesiq za mladi ucheni, HTMU, Sofia 19.05.2010*;* [2] M27-A2 „Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Second Edition”*;* [3] M38-P, Vol.18 No3 “Reference method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard.*;* [4] Antimicrobial Agents and Chemotherapy, January 2010, p. 1–23 Vol. 54, No. 1 *;* [5] Pearson et al.,Antimicrob. Agents Chemother. **18:**699–708, 1980

## MM17

# COMPARISON THE FUNGICIDAL PROPERTIES OF SILVER NANOPARTICLES SYNTHESIZED VIA CHEMICAL AND THERMAL REDUCTION OF SILVER NITRATE IN THE PRESENCE OF DIFFERENT POLYMERS AS STABILIZING AGENTS

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**Introduction:** Silver nanoparticles were synthesized using two different strategies. The first strategy for preparation of silver nanoparticles consists in chemical reduction of silver nitrate ($AgNO_3$) as a precursor of silver ions and

$NaBH_4$ as a reduction agent in the presence of polyvinylpyrrolidone (PVP) as a stabilizing agent. The concentration of silver was 19.6 mg/ml [1]. The second strategy consists in thermal reduction of $AgNO_3$ at 100 °C in the presence of polyvinylalcohol (PVA) as a stabilizing agent. The concentration of silver was 3.9 mg/ml.

**Aim:** Comparison the fungicidal properties of silver nanoparticles synthesized via chemical and thermal reduction of silver nitrate in the presence of different polymers as stabilizing agents.

**Materials and methods**: The synthesis of silver nanoparticles was confirmed by UV/vis spectroscopy. The fungicidal properties of thus prepared silver nanoparticels were further tested [2]. The presence of fungicidal activity of silver nanoparticles prepared by both techniques was confirmed and the Minimal Fungicidal Concentrations (MFC) of different control stains Candida spp as C.albicans ATCC 10231, C.tropicalis B 6030413, C.krusei ATCC 6258, C.glabrata ATCC 90050 and of the A.brasiliensis ATCC 16404 were estimated.

**Results**: The silver nanoparticles synthesized by the first strategy showed MFC for C.albicans and A. brasiliensis – 4.9 mg/ml, for C.glabrata and C.tropicalis - 2.4 mg/ml, and for C. krusei MFC was - 0.15 mg/ml [2]. The MFCs for the silver nanoparticles synthesized by the second strategy were significantly lower. For C.albicans and C.krusei MFC was lower than 0.03 mg/ml, for C.glabrata and C.tropicalis was 0.25 mg/ml, and for A.brasiliensis was 1.96 mg/ml.

**Conclusion**: Obviously, the manner of reduction of silver ions to silver nanoparticles plays an essential role on their fungicidal properties.

**References**: [1] Stanislav Nikolov, Rayna Bryaskova, Daniela Pencheva , Antibacterial hybrid materials based on polyvinilpyrrolidone whit embedded silver nanoparticles, poster in the VII scientific session of youth scientists, University of Chemical Technology and Metallurgy, Sofia 19.05.2010; [2] Daniela Pencheva , Todor Kantardjiev, Rayna Bryaskova, Fungicidal activity of hybrid materials based on polyvinilpyrrolidone whit embedded silver nanoparticles, accepted as poster to participate in the 12th Congress of the Bulgarian Microbiologist with international participation, Oktober 11-15, 2010, Yundola, Bulgaria

## MM18

# QUALITY TESTING OF COMMERCIALLY PREPARED ("BUL BIO – NCIPD") MICROBIOLOGICAL CULTURE MEDIA, ACCORDING TO THE REQUIREMENTS OF ISO/TS 11133

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**Introduction:** Culture media are used in all routine technics of cultivation and identification of microorganisms. Most formulas of culture media are commercially known and are described in literature. The reproducibility of the results obtained in microbiological laboratories depends first on the quality of culture media which requires repeatability of the data of growth promotion test for all lots of a given culture media. The growth promotion test of culture media is a basic requirement in many ISO standards concerning GMP (Good Manufacture Practice) and GLP (Good Laboratory Practice) as well as the sterility test of sterile and not-sterile products in European Pharmacopoeia.

**Aim:** Quality testing of different lots of solid culture media determining the Productivity ratio ($P_R$) and the Selectivity factor ($S_F$).

**Materials and methods**: Culture media: TSA "BD"as a referent media, soya-bean casein digest agar (SCA) „BB", Mueller Hinton agar "BB", Mueller Hinton agar with 5% sheep blood „BB", Saburo „BD"as a referent media , Saburo „BB", Saburo with chloramphenicol "BB", Candida chrom agar „BB", Mac Konkey agar „BB", Uri chrom agar „BB". Test microorganisms – suspensions standardized to 3 MF with „Densi La Meter": C.albicans ATCC 10231, C.tropicalis B 6030413 , C.krusei ATCC 6258 , C.glabrata ATCC 90050, B.subtilis ATCC 6633,P.aeruginosa ATCC 27853, E.coli ATCC 25922, S.typhimurium ATCC14028, Shigella flexneri NCTC 9950, Klebsiella pneumoniae ATCC 13883 , S.aureus ATCC 25923, S.pyogenes ATCC 12344, A.brasiliensis ATCC 16404 . The productivity of different lots of SCA and Saburo agar, produced by "Bul Bio – NCIPD" is compared to that of TSA and Saburo agar "Becton Dickinson". The lots of SCA and Saburo agar, produced by "BB"which productivity ratio respond to the $P_R$ norm (ISO/TS 11133: ≈ 0,7) were used to determine $P_R$ and $S_F$ of other solid culture media, produced in "Bul Bio-NCIPD".

**Results**: The following dilutions of test microorganisms were determined as appropriate to be used for inoculation of solid culture media, using surface agar method to calculate $P_R$ and $S_F$: $10^{-7}$ for E.coli ATCC 25922, S.typhimurium ATCC14028, S.flexnery NCTC 9950 and P.aeruginosa ATCC 27853, $10^{-6}$ for Klebsiella pneumoniae ATCC 13883 and S.aureus ATCC 25923, $10^{-5}$ for Candida sp., B.subtilis ATCC 6633 and S.pyogenes ATCC 12344 and $10^{-4}$ for

A.brasiliensis ATCC 16404 (seven days culture).

**Conclusion**: All tested lots of culture media, produced by "BB" meet the requirements for $P_R$ and $S_F$ of ISO/TS 11133.

## ММ19

# ТУЛАРЕМИЯТА В БЪЛГАРИЯ

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Доказването на туларемията в България е осъществено преди 50г. - декември 1960г. В района на езерото Сребърна – Силистренско. При ловуване на ондатри от намерени умрели и уловени живи е изолиран причинителя – *Francisella tularensis*, а при заболели хора е установена положителна серологична проба- аглутинация. В продължение на няколко години след това се установяват положителни резултати, което показва трайно поддържане на инфекцията в района на езерото. Във връзка с това в България беше разработена програма , проведени лабораторни изследвания, вкл. приготвяне на диагностични препарати – антигени и серуми и подготовка на специалисти за осигуряване на такива изследвания в страната.

През 1997г. при възникване на епидемичен взрив на туларемия в района на запад от гр.София, същата беше разпозната сравнително бързо и бяха проведени успешно комплексни диагностични и профилактични мерки, което продължава и до сега. В продължение на повече от 10 години са установени няколко десетки случаи на инфекцията в различни селища.При проведените лабораторни изследвания е изолиран причинителя от диви зайци, мишевидни гризачи, кърлежи, вода и хора, което потвърждава наличието на ензоотична и епидемична туларемия с трайно поддържане на инфекцията в Западна България.

През 2006г. в Североизточна България /Русенско и Шуменско/ отново беше установена туларемия сред диви зайци и заболяване на хора, което беше потвърдено с микробиологични и серологични изследвания. Още през 1952г. в този район- Тутракан са установени клинично заболявания на хора с лимфаденити след ухапване от кърлежи, което се приема за туларемия / не е потвърдено лабораторно/. Тези данни говорят за възможност за поддържане на инфекцията или периодичен внос на същата. В съседните на България страни /Турция, Румъния,Сърбия и Косово/ е установено наличието на туларемия. Всичко това показва необходимост от мерки за епидемиологичен контрол и надзор в страната и сътрудничество

със съседните и други страни. Тази необходимост е обсъждана на последната Шеста международна конференция по туларемия в Берлин- 2009г.

## ММ20

# СТО ГОДИНИ ОТ ИЗОЛИРАНЕТО НА ПРИЧИНИТЕЛЯ НА ТУЛАРЕМИЯТА

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Заболявания с клинико-епидемиологични белези характерни за туларемията са описвани преди откриването на причинителя и в Европа, Северна Америка и Япония и са описвани като чумоподобни, заешка треска и кърлежова треска.

Установяването на причинителя *Bacterium tularense* става по време на провеждане на изследвания на диви гризачи за чума през 1910г. в областта Туларе в Калифорния по повод възникване на епидемия от чума в Африка. В хистологични препарати от органи на *Cytellus beecheyi* McCoy и Chapin наблюдават полиморфни микрококи /кокобацили/. Заболяването наричат чумоподобно, а причинителя- Bacterium tularense . Опитите за култивиране на този причинител върху използваните по това време в микробиологията хранителни среди дълго време са неуспешни. Култивирането става чрез биопроба на морски свинчета в продължение на две години до прилагането на новата специална хранителна среда- яйчно-жълтъчна.

След публикуването на тези резултати- 1912г. тази среда се прилага успешно първо в САЩ, а по-късно в Европа и Япония. Като използва тази хранителна среда за осигуряване на чиста култура E. Francis провежда опити за приготвяне на агарова среда с различни комбинации от хранителни препарати. Това също продължава няколко години – през второто десетилетие на миналия век. В комбинация на пептон /дивко/, цистеин и заешка кръв /серум/ /ГЦБА/, известна като среда на Francis се постига изолирането на чисти култури. Тези резултати са публикувани в края на 20-те години. Двамата автори работят в различни лаборатории – Вашингтон / McCoy/ и Ню Йорк / Francis/. Двамата проявяват ерудираност, която осигурява постигането на тези резултати, които позволяват след 1920г. сигурна микробиологична и серологична диагностика на туларемията, което осигурява нейното успешно диагностициране в различни страни.

Приносът на двамата изследователи е оценен в медицинските среди и названието Туларемия се запазва, а за причинителя е прието название на

името на Francis – Francisella tularensis. Това е добър пример за признателност от научната общност, работеща в тази област.

# VETERINARY MICROBIOLOGY

## VM1

## RAPID METHODS FOR DETECTION AND QUANTIFICATION OF FOOD-BORNE PATHOGENS – IMPLICATIONS FOR RISK ASSESSMENT

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Different pathogenic bacteria are most commonly reported as causative agents of food-born infections in humans throughout the world. Traditional methods for detection and quantification of emerging zoonotic bacterial pathogens are slow and tedious. Therefore development of specific, sensitive and rapid methods are needed to collect sufficient data for risk assessment and food safety policy.

While the application of risk assessment and the HASSP strategy in combination with the knowledge of microbial ecology, offer the most cost-effective and reliable strategy for food safety management, there is still a need for methods to detect and enumerate pathogens, whether to develop information needed to construct predictive models for decision support and to verify them.

Molecular methods such as PCR-DGGE, real-time PCR, and microarrays allow either, detection and quantification of a target into a food matrix, or characterization of contaminating bacteria being still a subject of improvement. Modern methods to detect pathogens in foods are required by government authorities, industry and researches for trade, and for public health protection and surveillance. In all cases the methods must produce accurate, repeatable and reproducible results.

What are the needs and limitation of microbial food testing in 2020? Will we still use agar plates with supplements, enrich microbes and isolate them in time-consuming procedures? Reliable, timely detection and quantification of microorganisms in food remains a challenge to effective microbial food safety management.

**VM2**

# ZOONOTIC DISEASES AND THE ROLE OF HORSES AND OTHER EQUIDS AS ITS SOURCE

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The aim of this issue is to increase awareness and knowlege of the diseases of equids that have zoonotic potential, to review their relative public health significance, and to provide information on selected equine diseases for which specific safeguards are needed to minimize risk of transmission to humans in Bulgaria. An estimated 70 percent of infectious diseases of domestic and wildlife species has zoonotic potential and can, under certain circumstances, be transmissible to people. Notwithstanding the diversity of infectious agents involved, relatively few, however, are derived from horses or other members of the family *Equidae*. Most of the more frequently encountered zoonoses are contracted through direct or indirect human contact with other domestic species/wildlife. The range of diseases affecting the horse with zoonotic potential includes viral, bacterial, rickettsial, anaplasma, fungal and parasitic infections. Certain of these are listed by the International Office for Epizootic Diseases (OIE) as specifically equine diseases in that they normally do not affect other domestic species for examples - equine viral encephalomyelitides (Venezuelan, Eastern and Western equine encephalomyelitis), glanders and more recently, acute equine respiratory syndrome caused by Hendra virus.The second group is of infectious diseases that are listed by the OIE as multispecies diseases and which can affect various domestic/wildlife species besides the horse for examples - WNF, rabies, salmonellosis, anthrax and leptospirosis.

Occurrences of zoonotic diseases such as West Nile virus infection and avian influenza in recent years serve to underscore the need for greater cooperation between human and animal medical science if more effective prevention and control of these diseases is to be achieved.

## VM3

# SEQUENCING ANALYSIS OF GLICOPROTEIN C GENE OF SOME BULGARIAN ISOLATES OF CapHV 1

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The aim of this study is to establish if there are the differences in nucleotide and amino acid sequences in a section of the virus and strain specific glycoprotein C (gC) gene of different strains CapHV 1. We included two Bulgarian isolates from goats - Suhindol and Troyan, two from bucks - Biser and Kyustendil and two reference strains E/CH and Ba1 in the study. To carry out the PCR reactions we used in gradient purified DNA and one pairs of primers for the gC gene. Sequencing data from 350 bp have processed by MEGA4 software. In data analysis we have found presence of variable zone in Kyustendil, Suhindol and Trojan strains (position 887) and two in Biser (positions 887 and 1000). Based on these results evolutionary relationships and pairwise distance have been identified.

## VM4

# PREVALENCE OF TULAREMIA AMONG DOMESTIC ANIMALS IN SOFIA REGION

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A series of seroepidemiological investigations about prevalence of tularemia among domestic animals in Sofia's region was carried out. Two thousand one hundred sixty eight blood samples from cattle, sheep, goat, swine and horses were analyzed as well as a sera from homeless dogs.

During the period of seven years it was fined out 30.37% seroprevalence of tularemia among cattle population and 37, 57% among sheep and goats respectively. The seroprevalence in horse's population was determined to 83.78

%. A specific antibodies against *F. tularensis* in the sera of homeless dogs were proved in 23.96% of the samples. In the region of Bankya the tularemia was clinically expressed.

## VM5

## COMPARISON OF THE METHODS FOR THE TUBERCULOSIS PROOF BY THE FARM ANIMALS

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The routine diagnostic methods (pathoanatomically, microscopic, bacteriological and biological) are comparison with polymerase chain reaction (PCR). Throughout four years are investigated tissue suspensions from lymph nodes (111), lung (32), liver (19), as well as milk (10), nasal secrets (10) and blood (10) from tuberculin test doubtful and positive reacted cows. The percentage of coincidence between detached routine methods and PCR was respectively PCR/BIO – 100%, PCR/PA – 98%, PCR/BAC – 96% и PCR/MI –88%.

The received results showed that the PCR sensitivity coincided 100% with biological method and was more sensitively of the remaining standard methods. The PCR input can abolish the bioassays using. While considered the reaction rapidity, the using possibility of PCR as additional diagnostic method, that can improve and speed the tuberculosis diagnostics, is conclusively demonstrated.

## VM6

# ENHANCED RESISTANCE AGAINST SALMONELLA TYPHIMURIUM INFECTION IN CHICKEN, TREATED WITH A BACTERIAL PRODUCT (BP)

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**Introduction:** Certain bacteria such as mycobacteria, propionibacteria, nocardia and bacterial products, have long been known to possess powerful immunomodulatory properties. The interest to this immunomodulators was increased.

**Aim:** The aim of the study was to establish the effect of bacterial product (BP) on the immune response of chicken by infection with *Salmonella typhimurium.*

**Material and methods:** BP comprising different bacterial strains *S. typhimurium* is applied during 10 subsequent days in a dose of $1x10^9$ killed bacterial cells per os to 4-day old chickens before infection with *S. typhimurium.* The phagocytic activity was determined by the method described by Osada et al. Determination of the effect of BP treatment of superoxide radical and hydrogen peroxide production by leucocytes and macrophages was performed using the methods of Leslie and Pick. We used the hemagglutinating antibody assay.

**Results:** A protective effect, represented as a decrease in the number of the dead chickens during acute infection with salmonellas in comparison with untreated controls, has been established. The phagocytic activity of the leucocytes in the treated with BP and infected with salmonella chicken is increased. The production of superoxide species ($O_2$ and $H_2O_2$) responsible for the activity of the phagocyting cells is elevated. BP increases the synthesis of antibodies with a defined maximum at the $15^{th}$ day after *S. typhimurium* infection. An increase of the lymphocytes (B-cells) and hyperplasia on lymphoid follicles on bursa of Fabricius on the $15^{th}$ day, due to the greater biosynthesis of antibodies, is observed.

**Conclusion:** The elevated nonspecific and specific defense mechanisms are important to diminish the number of salmonellas and restrict their proliferation in the chickens' organs. The results obtained show that BP possesses a defined immunostimulatory effect and applied in a suitable dose and scheme increases the defense of the chickens.

# INFECTIOUS IMMUNOLOGY

## II 1

## IBOGAINE CONFERS PROTECTION IN MURINE *CANDIDA ALBICANS* SEPSIS

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The dimorphic fungus *Candida albicans* is the major pathogen that causes opportunistic infections called candidiasis. In the field of antifungal therapy, the introduction of new agents is permanently needed, because of the increased population of immunocompromised individuals, predisposed to opportunistic infections. In the present work, the indole alkaloid ibogaine was tested in *Candida albicans* sepsis induced in normal and indomethacin-suppressed mice at a dose of 5 mg/kg from day 0 to day 4 of infection. The substance decreased the rate of mortality, reduced organ dysfunction and histopathological changes in liver and kidneys. The positive effect correlated with an increase of serum IL-12 and α1-antitrypsin levels and a decrease of IL-10 level. The established inhibition of the process of glycosylation by ibogaine might have a protective effect on the course of *C. albicans* infection through embarrassed organ contamination. Although, we observed a specific antibody response against *Candida* in infected animals, ibogaine did not influence its level. The detected increase of plasma creatinine and bilirubin levels in mice non-treated with ibogaine, pointed on kidney and liver dysfunction. The alkaloid to a great extent reduced the impaired functions of these organs. As it is evident from the present results, creatinine level was close to normal and bilirubin level was significantly lower than in infected animals. Also, untreated with the substance mice had a greater liver and kidney pathology. It diminished the changes in tubular and glomerular parts of kidneys and reduced necrosis in liver. In conclusion, ibogaine possessed protective effect in *C. albicans* sepsis after a short course of application. It can limit further organ contamination and failure. Under the scheme used, ibogaine was ineffective in indomethacin-suppressed mice.

**II 2**

# ANTIGENIC PROPERTIES OF *YERSINIA* OUTER MEMBRANE PROTEINS

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*Yersinia enterocolitica*, *Salmonella enteritidis*, *Shigella flexneri* and *Campylobactor jejuni* in bowel infection, and *Chlamydia trachomatis* in urogenital infection are microorganisms which are known to induce reactive arthritis (ReA). Followup studies in patients with *Yersinia*-induced arthritis have shown that *Yersinia*-specific antibodies can be detected for months or even years in the serum of ReA patients. In this study *Yersinia* outer membrane proteins (Yops) are used as the antigens in *Yersinia* specific immunoblot assay. We established the presence of strong antibodies response against the protein antigen in 160 blood sera samples of patients with infectious arthritis. Anti-*Yersinia* IgG antibodies were established against YopH, YopM, YopB, V-ag, YopD, YopN, YopP and YopE. IgA antibodies were detected against YopB, YopD and YopN. IgM antibodies were detected against YopD. Antibodies of the IgM directed against YopD indicate an acute infection. The specificity of immunoblot analysis for IgM antibodies against Yops is 97%. These results suggested the specificity of antigenic properties of YopD in anti-*Yersinia* response.

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## II 3

# APPLICATION OF TYRPHOSTIN AG-490 IN LPS-INDUCED SHOCK

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Tyrphostins are synthetic family of compounds which are potent and specific tyrosine kinase inhibitors. Due to the importance of kinases in many cellular signaling pathways, including those leading to cell proliferation, and inflammation, the tyrphostins have been extensively studied as possible therapeutic agents. Excess amounts of LPS can cause endotoxic shock with high mortality. Because many cytokines have been shown to signal through JAK/STAT pathways, we suggest that AG-490 may inhibit cytokine-based immune deviation in sepsis. In the present study we have examined the liver and spleen pathology in mice with LPS-induced shock treated or not with AG-490. Furthermore, peritoneal cells were isolated at certain time intervals after LPS administration and tested for the expression of C5aR, TNFαR, CD3, CD69, Ly6C and CD11b markers. Our observations showed that AG-490 inhibited histological changes in liver and spleen concerning the influx of inflammatory cells. The percentage of C5aR expressing peritoneal cells slightly increased within 1h of LPS+AG-490 administration than in mice which received LPS alone as the increase was more significant at 4 h and at 24 h. The expression of C5aR was higher in LPS treated mice. Peritoneal cells obtained from LPS+AG-490 mice expressed higher levels of CD11b/Ly6 markers at 1 h followed by considerable decrease of CD11b expression at 4 h and 24 h. The expression of both markers in LPS treated mice was greater at 24 h. The results suppose that through C5aR and CD11b signaling tyrphostin AG-490 can ameliorate the rate of inflammation during LPS-induced shock.

**II 4**

# 7-HYDROXYCOUMARIN INFLUENCES THE IMMUNE RESPONSE OF CHICKENS INFECTED WITH *SALMONELLA TYPHIMURIUM*

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**Introduction:** 7-hydroxycoumarin (7-OHC) is the main metabolite of coumarin (1,2-benzopyrone) and is used for treatment of experimental animals and patients with tumors.

**Aim:** The aim of the investigation was to evaluate the effect of 7-OHC on the immune system of chicken and the protection against lethal *Salmonella* infection.

**Material and methods:** We have applied 7-OHC to 4-day old chickens per os at a dose of 50 mg/kg during 10 consequent days and after that have infected the chickens with *S. typhimurium.* We have infected in a series of experiments chickens with lower doses of two strains *S.typhimurium*: a virulent strain and an attenuated one with weak virulence. The phagocytic activity was determined by the method described by Osada et al. We used the hemagglutinating antibody assay.

**Results:** We have observed an increased survival at the $6^{th}$ and $8^{th}$ day after infection with a virulent strain, in comparison with control non-treated chickens.. Upon application of 7-OHC, as well as after infection with salmonella, the phagocytic activity of the leucocytes is increased. Ten days after the infection with the attenuated strain we have found increased biosynthesis of antibodies. The preparation does not show pathohistological changes in the chickens' liver when applied at the mentioned above doses and schemes, which proves its nontoxicity.

**Conclusion:** The defined protective effect is due most probably to the immunostimulation properties of 7-OHC.

# PLANT AND SOIL MICROBIOLOGY

## PSM1

## GENETIC DIVERSITY OF WHEAT DWARF VIRUS IN EUROPE

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**Abstract**

Cereal-infecting geminiviruses were one of the frequently isolated and caused huge losses during the last decade in several states of Europe. The presence of a wheat adapted and a barley adapted strain of *Wheat dwarf virus* (WDV) has been confirmed in the field samples. The complete genome of wheat (6 isolates) and barley (5 isolates) strains of WDV from Bulgaria, Hungary and Ukraine were isolated, sequenced and compared with available WDV sequences. The genome was found to be 2750 nucleotides long for wheat adapted and 2734 nucleotides for barley adapted WDV. The complete genome of barley strain isolates share around 80 – 85 % nucleotide identity with wheat strain isolates of WDV. WDV isolates of barley strain are more variable than the isolates of wheat strain. It is generally accepted, that barley strain is restricted to barley host, while wheat strain is present in both wheat and barley plants. Exception is the WDV-Odesza isolate which belongs to barley strain in spite of being isolated from wheat plant. In phylogenetic analyses, the WDV isolates formed two distinct branches, barley and wheat strains, which could be divided into two clades.

**PSM10**

# IN VITRO ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF DIFFERENT SPECIES AND STRAINS MICROALGAE

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**Abstract**

The microalgae possess varied biosynthetic possibilities and they could be cultivated in controlled conditions. The microalgae are a source for obtaining of biomass and medicine products.

The antimicrobial activity of different liquids water extracts obtained from microalgae species Chlorella, Gloeocapsa, Trachydiscus, Nostoc, etc. was investigated against the Gram-positive bacteria *Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus* as well as the Gram-negative *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Yersinia enterocolitica*), and *Candida albicans*.

Water extracts of genus *Gloeocapsa* algae showed activity against *S. aureus* and *S. pyogenes*. Comparatively the efficacy of ethanol extracts was stronger. The strongest activity was observed on lypophilic extracts from Trachydiscus minutus и Nostoc spp., dissolved in DMSO and/or in methyl alcohol, against *S. pyogenes*. Crude polysaccharid extracts from the genus Gloeocapsa act both against bacteria and fungi.

In conclusion it could be underlined that antimicrobial activity depends on the species of microalgae, conditions of their cultivation as well on the efficacy of extraction procedures.

**Key words: microalgae, antimicrobial activity**

**PSM11**

# MORFOLOGICAL AND BIOLOGICAL CHARACTERISTICS OF ENTOMOPATOGENICAL VIRUSES ( BACULOVIRUS) ISOLATED AND STUDIED IN BULGARIA

A. Atanassov, Kostinbrod

Accomplished is analysis of the isolated and studied in Bulgaria entomopatogenical viruses suitable for biological plant protection. Comparison are the their morphological characterizations. Indicate is the biologically efficacy against the pest insect hosts. Enumerate are the preparations development in Bulgaria an the basic or with involving of entomopathogenic viruses. Examine with the prospects for their use in the production of organic vegetable produce.

# FIRST AUTORS' INDEX