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# ADVANCES

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## ADVANCES IN BULGARIAN SCIENCE



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#### Editorial board:

Vanya Grashkina, Olga Racheva, Yana Panova, Kostadin Tonev, Milen Angelov, Tzvyatko Stoyanov Lyudmila Velkova

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### NATIONAL SCIENTIFIC PROGRAMS - AN INSTRUMENT FOR STIMULATION OF RESEARCH AND INNOVATION

National scientific programs in Bulgaria play the part of specialized priorities in the field of science, aiming at increasing the competitiveness of the national economy. They are approved as relevant to the European Framework Programs for development of science and technology and are in conformity with the requirements of the European Commission on reciprocity with regard to participation of other countries.

The main characteristics of the national programs are: creation of conditions for sustainable economic development; improvement of the environment for research and innovation; fostering the conditions for regional science and innovation development; improvement of the effective support of the research work; binding to the priorities of the European Union.

National scientific programs are expected to bind horizontally different national and regional scientific programs and initiatives and to create conditions for coherent environment between different national, regional, and European policies. An open model for coordination of scientific research and innovation as well as other activities connected with the development of the human resource is achieved through effective development of national scientific programs. Areas of competency are being defined, synergetic effect is being achieved, and technology clusters are being formed. The applicable instruments are from the thematically orientated programs in the priority fields for the country, via reciprocity of the national programs at regional and European levels up to building up joint program initiatives on the principle of mutually agreed priorities.

The topic of the present issue of "Advances in Bulgarian Science" is the national scientific program "Genomics".

Genomics is a new science concerning with the analysis of genetic information coded in the genomes of a living being, including those of a human being. It won recognition during the last decade in connection with the development of the international Human Genome Mega Project, the first stage of which was successfully completed in 2000. Genomics has disclosed unexpected prospects for deciphering of genetic information and using it in favour of a man.

During the recent years a Functional Genomics subprogram was started within the framework programs for scientific research of the European Union. Its aim is to finance strategic integral projects in the field of genomics, as well as to unite efforts and improve qualifications of scientists from European countries working in this field. In view of the strategic place of genomics for the development of medical, biological, agrarian sciences and pharmaceutics during the next decade, national programs on genomics have already been started in most of the European countries. Giving an account of the fact that Bulgarian science is a part of the European science, the national Genomics Program should be considered as a projection of the European program reflecting specific for Bulgaria problems and interests.

The aim of the program is to accelerate the development of genomics, using the existing and getting new knowledge on genomes of important for the country living organisms (bacteria, plants, animals and man). The efforts should be directed to creation of conditions for increasing the quality of the public health and life of the Bulgarians and preservation of biodiversity in the country. The program realizes its aims through integrated projects in genome analysis, bioinformatics and new genome markers.



#### MAIN OBJECTIVES

NACID is a governmental institution affiliated to the Ministry of Education and Science. NACID collects, processes, maintains and disseminates reference and analytical information to support the national policy in the field of education, science and innovation as well as to support Bulgarian research bodies, individual researchers and SMEs.

### PRIMARY FIELDS OF ACTIVITIES:

- > Processing and disseminating bibliographic, reference data and analytical information.
- > Maintaining specialized databases of scientific production and research resources in Bulgaria.
- Providing information about national, European and trans-European research programs.
- > Providing information to support the process of harmonization of the Bulgarian education and research legislation with European Union ones.
- > Performing the role of institutional contact point of the Sixth Framework Program in Bulgaria.

### INFORMATION PRODUCTS AND SERVICES

□ NACID offers a large range of information products, including:

- Subject profiles;
- Reviews;
- Bibliographic references;
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- > "Who is Who in Bulgarian Science"-more than 5 500 records
- ▶ "Papers" more than 120 records
  - "Science and Industry" Databases
- > "Partnership for Innovation and Development" information about the national Research units.
- > "Knowledge for Innovations and Development" information about Bulgarian R&D activities.

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### NATIONAL SCIENTIFIC PROGRAMMES WITH EUROPEAN DIMENSIONS

### PLANT GENOMICS RESEARCH IN BULGARIA - PRESENT STATUS, PROBLEMS AND DIRECTIONS OF DEVELOPMENT

Atanas Atanassov, Elena Marinova, Elena Todorovska, Ivan Atanassov, Rositza Bachvarova, Tzvetanka Hvarleva – AgroBioInstitute, Sofia, Bulgaria Aglika Edreva, Kostadin Gecheff – "Doncho Kostov" Institute of Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria Dimitrina Kostova, IZK "Maritsa", Plovdiv, Bulgaria Ilia Denev, Tsanko Gechev – Plovdiv University, Plovdiv, Bulgaria Ivan Todorov – Institute of Agriculture and Seed Science "Obraztsov Chiflik", Rousse, Bulgaria Natasha Kovacheva – Institute of Oil Rose, Kazanlak, Bulgaria Nikolai Tsenov – Dobroudja Agricultural Institute, General Toshevo, Bulgaria

The beginning of the 21st century marks the era of functional genomics. The genomics research is driving the development and rapid progress of a number of "omics" technologies directed to the analysis and deduction of gene (one or many) function in a short time, at the transcript (transcriptomics: parallel analysis of gene expression), protein (proteomics: analysis of polypeptide complement), and metabolite (metabolomics: parallel analysis of a range of primary and secondary metabolites) levels. As a result plant genomics leads not only to rapid expansion of our understanding of how genes determine plant function, but also promote consolidation of diverse research areas, bringing together biological, agricultural and ecological studies. The genomics studies is relating with substantial and elevating rate of funding, infrastructure development and advance training correlated with increasing international cooperation and concentration of research capacity. At the same time the level of performed genomics research largely determines the rate and capacity of nation-wide technology development, transfer and applications in large areas of agricultural and food industries. This is particularly important for countries like Bulgaria, possessing very limited resources for development of plant genomics. Thus, determine and implementation of adequate and successful mid- to long-term national directions and priorities of genomics research will be crucial for further development of Bulgarian agricultural and plant sciences as well as providing of the necessary support for implementation of advance practices for product quality control and "modern production" technologies.

### I. CURRENT STATUS AND PROBLEMS OF OR-GANIZATION, FUNDING AND PERFORMING OF PLANT GENOMICS RESEARCH IN BULGARIA

In 2001 the Ministry of Education and Science applied and the Council of Ministers selected six priority National Scientific Programs. Among them was "GENOMICS", relevant to EC FP6 priorities for scientific and technological development and considering traditionally good practices and research capacities of the country. The Decision was that funds would be annually covered by the Budget in the frame of group "Research" equally by the Ministry of Education and Science (National Science Fund) (NSF) and Ministry of Agriculture and Forestry (MAF).

The National program was launched in 2003. The main advantage of this program is to join the best for the moment researchers from different Bulgarian institutions in the field of plant genomics.

### Main achievements:

> Large scale genotyping of a number of crops and respective EU cooperation;

> Advanced and extended transgenic and GMO management researches;

Internet accessible Bulgarian plant genomics database;

Started advance extension services for GMD and genetic authenticity analysis;

Started first in the country genome wide mapping projects.

### Main problems:

> Insufficient rate of government-backed funding for genomics research;

> No clear vision and implementation of the long-term government-backed program for plant genomics;

> Still underdeveloped national industry and small demands for modern biotech products and technologies;

No infrastructure for "omics" technologies.

### The most urgent needs and measures:

> Establishment of "minimal" infrastructure for the application of "omics" technologies, providing countrywide services at competitive prices.

II. CURRENT PROJECTS SUPPORTED BY THE NSF, PROGRAM "GENOMICS"

II.1. MOLECULAR MARKERS FOR GENETIC AU-THENTICITY, MARKER ASSISTED SELECTION AND PRODUCTION OF CERTIFIED PLANTING MATERIAL FROM ECONOMICALLY IMPORTANT CROPS (wheat, grape, sunflower, rose)

### II.1.1. Partner: AgroBioInstitute - Sofia

**Program Module Leader**: Acad. DSc. Atanas Atanassov

*Key words:* molecular markers, wheat, grape, sunflower, rose, genotyping, QIL, marker assisted selection, mapping

The SSR - (Simple Sequence Repeast) markers are one of the most developed DNA markers and have been extensively exploited in genotyping and gene diversity studies. Recently, reference microsatellite maps have been developed from most economically important plant species and have been extensively used in the breeding programs. The SNP (Single Nucleotide Polymorphisms) markers are the next wave highly perspective di-allelic DNA markers that already have found multiple applications in plant genotyping, gene diversity studies, mutation detection analyses, QTL and MAS.

The main directions of studies proposed by the ABI research group in this multidisciplinary project are as follows:

> Genotyping of economically important lines and cultivars by SSR markers;

> Development of an experimental protocol for detection of SNP polymorphisms and its application in genotyping analyses of economically important lines and cultivars;

> Selection of core SSR and SNP marker sets sufficient for determination of genetic identity of economically important for Bulgaria crops;

> Characterization of the segregating mapping populations by SSR markers and mapping of loci for the important agronomic traits, Mapping of genes, specifically expressed in petals, and those responsible for important agronomic traits by SSR markers;

> Selection of appropriate SSR and SNP markers for MAS.

### II.1.2. Partner: Dobroudja Agricultural Institute - G. Toshevo

**Program Module Leader:** Associate Prof. PhD Nikolai Tsenov

Key words: wheat, sunflower, hybridization, abiotic stress, DNA, SSR-markers

Varieties and breeding lines of wheat and sunflower are included as the object of study. The aim of the project is an optimal organization of experimental research work on the use of molecular markers. The Laboratory of Molecular Biology is suggested to be equipped with the necessary consumables for DNA isolation. Analyses of this DNA are performed in ABI. The results obtained are processed simultaneously in both institutes via Internet.

The experimental research work on sunflower includes:

> Genotyping by 10 microsatellite markers of parental lines included in own and foreign hybrids;

> Development of a segregating population by crossing lines, which carry genes for resistance to downey mildew and broomrape;

> Analysis of the population by microsatellite markers;

> Genetic analyses of materials with regard to the microsattelite markers used.

The experimental research work on wheat includes:

> Genotyping by 30 microsatellite markers of promising breeding lines and economically important varieties;

> Developing of a segregating population by crossing lines resistant and susceptible to frost;

> Analysis of segregating population by microsatellite markers corresponding to loci comparatively evenly distributed in the wheat genome.

Within the project's framework it is expected to introduce efficient use of markers in breeding work as a result of establishment of the necessary conditions and organization for their application. The following practical results are expected:

> Determining markers, which characterize economically important lines and varieties;

> Determining the genetic identity of the planting material;

 $\succ$  Developing of method for quantitative analysis of the homogeneity of the wheat.

### II.1.3. Partner: Institute of Oil Rose - Kazanlak Program Module Leader: Natasha Kovacheva

Key words: oil rose, molecular markers, genotyping, cross-breeding, segregating populations, gene mapping.

The aim of this study is to genotype sorts from oil - bearing rose and to determine specific genes.

Cross-breeding materials including different genotypes are used. Methods of Sequence Repeats and Single Nuclear Polymorphism for evaluation and characterization of determined genes, distillations, gas-chromatography and organonoleptic analysis of oils are used.

### II.1.4. Partner: Institute of Agriculture and Seed Science "Obraztsov Chiflik", Rousse

Program Module Leader: Prof. DSc. Ivan Todorov

*Key words: V. vinifera,* inbreeding, hybridization, seedlessness, resistance, molecular markers

Having in mind fast development of elaboration and application of high effective molecular markers in plant genomics it is considered to form consortium connected with research and practical application of molecular markers to *Vitis vinifera*.

The main objectives of the consortium are as follows:

• Fast development and application of high technological methods for control and evaluation of quality and origin of planting material in agriculture;

• Routine application of molecular markers in selection of vine, starting with registration of lines and cultivars and intellectual property protection;

• Marker assisted selection of polygenian symptoms conditioned by great number of loci/genes.

The following activities take place in IASS "Obraztsov Chiflik" Rousse:

• Maintenance and phenotype characterization of collections of lines and vine cultivars;

• Passportization of cultivars and vine forms on the basis of molecular markers;

• Joint research with ABI on: (i) genotyping of collections of lines and cultivars on the basis of SSR and SNP markers; (ii) analyzing of segregation populations by SSR and SNP markers; (iii) processing of experimental results.

II.2. IDENTIFICATION OF KEY GENES CONTRIB-UTING TO THE INCREASED RESISTANCE OF IM-PORTANT CROPS AGAINST BROOMRAPE WEEDS (OROBANCHE SPP.)

**Project leader:** Ilia Denev, "Paisii Hilendarski" Plovdiv University, Plovdiv

#### II.2.1. Partner: AgroBioInstitute - Sofia

**Program Module Leader:** Dr. Rositza Bachvarova **Key words:** Genomic bases of plant pathology, new genomic markers, *Arabidopsis* mutants, differential display, *Orobanche* spp., resistance

It is well known that broomrapes (Orobanchaceae) are obligatory root parasites of plants. About 70% of agricultural lands in Southern Europe and Mediterranean are infested by several broomrape species, which parasitize important crops, thus causing tremendous agricultural losses. Currently there are no effective, inexpensive control measures for broomrape that can be applied in case of large scale outbreak. Genetic engineering of resistance mechanisms is the most promising solution, but it requires knowledge of fundamental aspects of host-parasite interaction that is currently lacking. The project aims to focus investigations on haustorium penetration, because it precedes parasitism and accompanying crop damages. Arabidopsis has been chosen as a host for Orobanche, because of its sequenced genome and readily available mutant populations. Several lines with enhanced resistance have been selected. Differential display to identity genes involved in host-parasitic interactions both in resistant mutants and wild-type hosts have been used. Results are expected for starting the characterization of resistance mechanisms. All genes identified as contributing to the host-parasite interaction will be characterized as to copy number and expression pattern. The presence of related genes in economically important Orobanche host plants will be determined.

### II.3. MOLECULAR MARKERS FOR QTL ANALY-SIS OF FUNGAL DISEASE RESISTANCE FACTORS AND IMPORTANT AGRONOMIC TRAITS IN GRAPEVINE

**Project leader:** Acad. DSc Atanas Atanassov **Program Module Leader:** Acad. DSc Atanas Atanassov

Key words: grapewine, fungal diseases' resistance, segregating population, molecular markers, genome mapping, QIL-analyses, metabolite analysis

Grapevine is an economically important crop in Bulgaria, but also with growing requirements for the quality of production. The development of grapevine cultivars resistant to fungal pathogens (powdery mildew, downy mildew and grey mould) is of great importance for both improving of viticulture and wine quality and production as well as reducing of fungicide4 spray volumes. The cultivar Storgozia created in the last century combines resistance to fungal diseases and high quality of grape and wine. The established segregation population, obtained after self-pollination of plants of cultivar Storgozia, gives unique possibility for direct identification of QIL locies conferring resistance to fungi and economically important traits.

The proposed integrated project involves a complex (phenotypic, molecular and metabolic) characterization of the above-mentioned segregation population. Core mapping of the populations with microsatellite markers, combined with phenotypic and metabolic analysis of the plants, will provide identification and characterization of QTLs related to the fungal resistance and important agronomic traits, as well as selection of the range of molecular markers linked to the QTLs. The obtained results will be further incorporated into the respective breeding programs. The project development will provide an excellent opportunity for initiation of complex genomics research in the county, focused on the pressing problems of Bulgarian agriculture and industry.

II.4. GENETIC VARIABILITY IN MUTANT BAR-LEY LINES INDUCED BY IONIZING RADIATION *Project leader:* Prof. DSc. Kostadin Gecheff, Institute of Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria

### II.4.1. Partner: AgroBioInstitute, Sofia Program Module Leader: Dr. Todorovska, Dr. Elena

Georgieva

Genetic diversity achieved by interspecies crosses or by induced mutagenesis underlies improvement of the plant species. Effective use of experimentally induced mutants in breeding programs depends a lot on the nature of the induced mutations. The main goal of the project is to reveal the cytogenetic and molecular nature of ionizing radiation-induced mutational alterations in a stock of originally produced mutant barley lines. Three main assumptions for the genetic nature of the radiation-induced mutational changes of breeding value are put forward in this project: these changes are due to point (gene) mutations; they are linked with the induction of structural chromosomal alterations; such type of changes is provoked by chromosome breaks in the vicinity of the genes, responsible for the trait

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of interest. The expected results are going to contribute towards elucidation of one important but underestimated problem, concerning the character of the initial mutation events induced by ionizing radiation in plant genome and for better and directed utilization of genetic variability in the contemporary breeding practice.

II.5. MOLECULAR BIOLOGY AND GENETICS OF PROGRAMMED CELL DEATH IN ARABIDOPSIS THALIANA, FNSF-2018, 2005 - 2006,

Project leader: Dr. Tsanko Gechev

**Keywords:** PCD hydrogen peroxide, AAL-toxin, transcriptome analysis

Programmed cell death (PCD) is a genetically controlled suicidal process in which cells selfdestruct upon certain cell death stimuli, selectively removing in this way unwanted cells. This process is an essential part of plant development as well as of many plant responses to abiotic stress and pathogens. Examples of developmentally controlled PCD can be found during development of the embryo, nucellar degeneration, maturation of tracheal elements, and epidermal trichomes, formation of lace leaf shape, and leaf senescence. A type of PCD is also the hypersensitive response (HR) - a defense reaction in which plant cells in and around the site of pathogen contact sacrifice themselves in order to physically restrict the spread of the pathogen. On the other hand, some necrotrofic pathogens can secrete toxins that trigger PCD in healthy tissues so that the pathogens can feed on the death tissues. Unwanted PCD can be caused also by some environmental factors like cold, salt, and heat stress. Thus, PCD is not only an essential part of plant development and beneficial for defense against pathogens, but can be an unwanted and even dangerous plant process. While the process is well studied in animals, little is known about the mechanisms and genes involved in plant PCD.

The main aim of the proposed project is to unravel the mechanisms behind the plant PCD by discovering genes involved in the regulation and execution of PCD in the model species Arabidopsis thaliana.

To realize that goal two experimental approaches will be used: (1) forward genetics, in which we will screen for mutations in genes that render plants altered in PCD responses, and (2) expression

profiling of PCD coupled with reverse genetics, in which by extensive microarray analysis we will identify genes that are transcriptionally regulated during PCD and those genes will be functionally studied by the knock out technology or by generating plants with altered expression of the genes.

Three PhD students will be assigned to carry out the experiments in order to develop highly qualified young Bulgarian specialists in the field of Genomics.

### II.5.1. Partner: Plovdiv University 'Paisii Hilendarski", Plovdiv

Program Module Leader: Dr. Tsanko Gechev

Programmed cell death (PCD) is a genetically controlled suicidal process in which cells selfdestruct upon certain cell death stimuli, selectively removing in this way unwanted cells. This process is an essential part of plant development as well as of many plant responses to abiotic stress and pathogens. Examples of developmentally controlled PCD can be found during development of the embryo, nuclear degeneration, maturation of tracheal elements, and epidermal trichomes, formation of lace leaf shape, and leaf senescence. A type of PCD is also the hypersensitive response (HR) - a defense reaction in which plant cells in and around the site of pathogen contact sacrifice themselves in order to physically restrict the spread of the pathogen. On the other hand, some necrotrofic pathogens can secrete toxins that trigger PCD in healthy tissues so that the pathogens can feed on the death tissues. Unwanted PCD can be caused also by some environmental factors like cold, salt, and heat stress. Thus, PCD is not only an essential part of plant development and beneficial for defense against pathogens, but can be an unwanted and even dangerous plant process. While the process is well studied in animals, little is known about the mechanisms and genes involved in plant PCD.

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Three PhD students will be assigned to carry out the experiments in order to develop highly qualified young Bulgarian specialists in the field of Genomics.

The ultimate goal would be integration of all data into a model explaining the com - plexity of plant PCD.

### II.5.2. Partner: Institute of Genetics, Bulgarian Academy of Sciences, Sofia

MODULE: BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF MUTANT ARABIDOPSIS THALIANA PLANTS ALTERED IN THE  $H_20_2$ -IN-DUCED PROGRAMMED CELL DEATH

**Program Module Leader:** Prof. DSc. Aglika Edreva **Key words:** Programmed cell death (PCD); Arabidopsis thaliana (wild type and mutants); reactive oxygen species (ROS); hydrogen peroxide  $(H_2O_2)$ ; enzymatic and non-enzymatic  $H_2O_2$ -scavengers; chlorophyll fluorescence

Programmed cell death (PCD), an integral part of life cycle, is relatively well understood in animal organisms, while in plants genetical and biochemical mechanisms underlying PCD remain still unclear. Data point to the key importance of reactive oxygen species (ROS) in induction and execution of PCD, a special role being attributed to  $H_00_{,}$ , a ROS of both signal and toxic properties. To gain a better insight into the biochemical events related to the regulation of PCD in plants (focusing on the role of ROS and particularly HO,), a reliable model system (Gechev et al. 2005) will be used. It is based on PCD induction by high endogenous levels of H<sub>Q</sub> in A. thaliana plants resulting from specific inhibition of catalase activity by aminotriazole (AT), whereas in A.thaliana mutants, although ATsupplied, PCD is compromised. Our hypothesis is that the suppression of PCD in A. thaliana mutants may be related to a default in HO, function or to depletion of H<sub>Q</sub> level due to interference by H<sub>0</sub>-scavenging factors. To validate the latter assumption, the content of  $\rm H_2O_2$  will

be determined, and the activity, molecular heterogeneity, and content of main components of enzymatic and non-enzymatic H\_0\_-scavenging network will be estimated: catalase, guaiacol peroxidase, ascorbate peroxidase, glutathione peroxidase, ascorbic acid, glutathione, etc. The importance of chloroplasts as a site of generation and scavenging of H<sub>Q</sub>, and the extremely scarce data on their role in PCD, the photosynthetic performance and the photochemical efficiency of photosystem II will be studied. Our hypothesis is that failure of PCD in A. thaliana mutants can be related to maintaining of a lower pool of ROS in chloroplasts through enhancement of alternative routes consuming excess electrons, such as photorespiration and Mehler reaction. Results of both fundamental and applied importance, contributing to a deeper knowledge of PCD in plants, can be expected.

### II.5.3. Partner: Institute of Vegetable Crops "Maritsa", Plovdiv

### MODULE: PHENOTYPE ANALYSIS OF PRO-GRAMMED CELL DEATH IN ARABIDOPSIS THALIANA

**Program Module Leader**: Dr. Dimitrina Kostova **Key words:** PCD, pathogens, plant viruses, hypersensitive response.

Programmed cell death (PCD) plays an important role during plant development and stress responses. The hypersensitive response (HR) is one of the most common forms of PCD. The purpose of HR is to physically restrict the spread of the pathogen and to generate signals that switch on the so-called systemic acquired resistance, a defense reaction in the distant cells and tissues.

While a significant advance was made in terms of receptor-pathogen interactions, still little is known about the genes and signaling cascades that lead to PCD.

The aim of the proposed project is to identify genes that are key regulators of plant PCD and plant response to pathogens. First, a largescale screening for mutants that are altered in PCD responses will be performed. Subsequently, those mutants as well as knock-out lines of genes implicated in PCD will be tested for resistance/sensitivity to plant pathogens. . . . . . . . . . . . . . . . . . .

### III. INTERNATIONAL EVALUATION AND REC-OMMENDATIONS

VIII<sup>th</sup> International Consultative Council (ICC) of AgroBioInstitute (ABI) pointed out that during the last three years markers were developed, based on microsatellites as well as AFLP, for the following crops: wheat, maize, barley, grape, small fruits, oilrose, sunflower and citrus.

Some of the research (e.g. that on grapes) was done with companies, predominantly for identification and breeding purposes. Research is done on a contract basis, which is important for the ABI because it provides financial support and permits publication. The latter is part of the contract but the company is allowed to patent (mainly Bulgarian patents). Improvement of the application of molecular markers was recommended to map QTL's for the purpose of marker-assisted selection (MAS).

Concerning the Genome Centre initiative of ABI it was stressed that In December 2000, plant genomics was recognized as a key priority at the milestone EC-supported Versailles Conference on Agricultural research in the European Research Area. The EC expressed serious concern about lagging behind colleagues and competitors not only from the USA, but also from China, Japan and even Brazil.

The ICC recognizes that the Genome Centre (GenomeC) initiative presented by the ABI properly highlights the importance of the knowledge and strategies for Genome research and technology application in the ERA-NET and the rest of the developed world, for example the USA and Japan.

The ICC found that its original recommendation for establishment of the Genome Centre during its last meeting (two years ago) had not moved beyond its primary conception. It was recognized that the current restrictive GMD law seriously hampers the establishment of the Genome Centre.

ICC also endorses the view that the priority of the Genome Centre should be to deal with regional species and breeds and that this should be done in the context of international collaboration.

ICC understands that the Genomics Centre as it is presented is expected to underpin and promote Bulgarian strategies and ambitions in the social and economic domain.

The development of a national program for Genomics entirely fits the government policy in which Genomics is one of the five priorities. The establishment of the Genome Centre is considered as an appropriate step in this direction. It aims to contribute to the efforts of Academia and the private sector. The establishment of such a key centre could lead to the generation of products and services with a significant economical, social and ecological impact. As such, the Genome Centre could:

• Create close synergy between research policy, innovation policy and educational policy in order to contribute to the whole Lisbon strategy for knowledge-based economy and society;

• Avoid duplication in genomics at the national level, bringing together life science from advanced laboratories, institutes, universities, and private sector laboratories;

• Accumulate a "critical mass" of state-ofthe-art specialized equipment;

• Develop and effectively implement hightech methods for diagnostics, control and assessment of quality and origin of food, feed and other products. This in turn should provide the means for preservation and protection of traditional Bulgarian species/breeds;

• Improve the synergy with the leading international research centres, companies and programs (e.g. with the ERA NET program and EMBO);

• Assure effective technological transfer and development of collaborative high-tech products.

On the basis of the above, the ICC makes the following suggestions:

### At the policy level:

• The ICC supports the efforts of the ABI, NCAS, ABT and BF of Sofia University to establish GenomeC in Bulgaria. For further successful development of plant genome research it is essential to have strong governmental support not only at the policy level, but also financially. The ICC particularly invites the Ministries of Science and Education, Agriculture and Forestry and Environment and Waters to reinforce their support to the GenomeC initiative.

• The ICC recommends that the GenomeC be used as an instrument for Bulgarian participation in international initiatives and that it strives to establish synergy between different scientific fields, education, technology and community. • All operations of the GenomeC should be carried out with the highest possible level of quality. The ICC therefore invites national and international bodies to financially support accreditation of the Genome Laboratory in the ABI. The ICC notes that this would align the GenomeC with similar laboratories in the EU.

The ICC notes that, at the R&D level, the following examples of research projects have particular importance for Bulgaria:

• Assisting breeding programs with molecular techniques for improvement of local cultivars and breeds;

• Production and distribution of control and reference samples for local cultivars and breeds to be used. Accreditation scheme for GMO detection and traceability, authenticity and phytosanitary status of valuable plants and breeds;

• The Genome characterization of the economically important local varieties and breeds could lead to understanding what is unique about their genomic background in order to provide information for biosafety purposes;

• Exploitation of the unique flora and fauna for production and use of products important for nutrition, pharmaceuticals, bioremediation etc.







### FAMILIAL COLORECTAL AND ENDOMETRIAL CANCER

Kadiyska T., PhD

Laboratory of Molecular Pathology, University Hospital of Obstetrics and Gynaecology, Sofia

### COLORECTAL CANCER

Colorectal cancer (CRC) is the most common gastro-intestinal neoplasia. The cumulative lifetime risk of CRC and mortality from CRC is approximately 5-6 % and 2,5 % respectively. The majority of colorectal cancers are sporadic (75-80 %). Approximately 20-25% are determined as familial CRC. Hereditary nonpolyposis colorectal cancer (HNPCC), or the Lynch syndrome is the most common type of familial CRC and accounts for approximately 1 to 5% of all colorectal cancers. HNPCC is an autosomal dominant disease with a high risk for colorectal and HNPCC- related cancers, caused by germline mutations in DNA mismatch-repair genes (MMR). These mutations lead to genomic instability, manifested by microsatellite instability (MSI). The screening for MSI might have prognostic implications. Patients with CRC whose tumors have high frequency MSI (MSI-H) have a more favorable prognosis, independently of the stage at diagnosis, than patients without MSI (MSS). In the treatment of CRC patients there are indications that MSI-H tumors may be resistant to the actions of certain commonly used chemotherapeutic agents such as fluorouracil, raising the important possibility that chemotherapy may have different outcomes in this group of patients. It has been found that the phenomenon of DNA instability is not confined to the setting of hereditary disease and may be seen in up to 15% of CRC in those with no family history of disease. In the case of these so-called sporadic tumors, mutation of MMR genes is relatively infrequent whereas biallelic hypermethylation of the promoter of MLH1 appears to be the most important mechanism for inactivation of MMR genes.

### ESTABLISHMENT OF A NATIONAL REGISTRY FOR COLORECTAL CANCER

In 1998 a National Registry for Colorectal cancer was initiated in Queen Giovanna Hospital, Sofia, Bulgaria. The medical records, blood samples and tissue specimens from 294 incident CRC cases diagnosed in the period 1998 - 2005 have been collected. Family information, including the main causes of morbidity and mortality of first and second-degree relatives, was recorded and genealogical tree prepared. A tissue and DNA CRC banks have been established in the Laboratory of Molecular Pathology.

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All patients received genetic counseling and informed consent was obtained for data and sample collection. Patients were sub-grouped into four main categories: 1. HNPCC families, according to Amsterdam criteria; 2. Families according to Bethesda criteria; 3. "Family" cases group - represented by families with one or more first-degree relatives of the proband affected by tumors at any site; 4. "Sporadic" cases whose families did not show any other cancer cases among relatives or without sufficient family information.

The clinical and histological information recorded includes patient's age at diagnosis, tumor localization, tumor stage and differentiation, presence of synchronous or metachronous tumors.

The following four years the research project supported by the National Science Fund aimed to initiate the National Registry and to involve several genetic methods in the molecular diagnostics of CRC: "Investigation of possibilities for molecular diagnostics and prophylactics of hereditary colorectal cancer in Bulgaria". The work was further extended by a new two - year project " Investigation of the molecular genetics and clinical profiles of patients with hereditary colorectal cancer in the Bulgarian population". The aim of the project was to improve and to expand the National Registry and the possibilities of early diagnostics and prophylactics.

### Microsatellite instability analysis

In 2001 one-year research project "Microsatellite instability analysis in Bulgarian patients with Colorectal Cancer" funded by the Science Fund of Medical University-Sofia aimed to analyze the prevalence and the role of genetic instability testing in the molecular diagnostic strategy. The next two years two other projects supported by the National Science Fund continued the study of MSI and its correlation with clinical and pathomorphological characteristics. Recently one-year research project continued this work with the collection of new CRC cases.

A total of 150 patients with colorectal cancer have been included in the study. DNA from fresh blood and/or frozen normal and tumor tissue was isolated using standard protocols. A set of five polymorphic markers- BAT26, D2S123, D5S346, D18S35 and FGA has been selected for analysis of MSI. The repeat markers were amplified from both normal and tumor DNA samples and electrophoresis was performed on an automated fluorescence sequencer (ALF Express, Pharmacia).

We detected 25 tumours with MSI and 14 with loss of heterozygosity (LOH) in at least two of the loci analyzed. The MSI phenotype was associated with distinct clinicopathological features, notably proximal tumor site, high grade and mucinous production. The LOH tumors were more frequently distally localized and no correlation was found according to the grade and mucin production. No difference was seen in sex, age of onset and staging, presence of synchronous or metachronous tumours.

### Analysis for MLH1 promoter hypermethylation

In 2001 the National Science Fund supported the two-year research project for young scientists "Investigation of promoter methylation of MLH1 and MSH2 genes on the microsatellite instability in colorectal cancer".

In 25 MSI positive tumors (10 sporadic cases and 15 with family history), 13 with loss of heterozygosity (eight sporadic cases and five with family history) and 15 random selected sporadic MSInegative cases, the methylation status of the MIHI promoter has been analyzed using polymerase chain reaction (PCR)-based HpaII restriction assay. Significant association was found between this trend and MSI/LOH (p<0.04). The correlation between the MIHI promoter methylation and the MSI sporadic cases was highly significant (p<0.01). In this group there were no patients younger than 50 years (p<0.02). The correlation between MIHI promoter methylation and the LOH sporadic cases was also significant  $\left(p\!\!<\!0.02\right)$  .

Our results supported the role of MIH1 promoter methylation as a major mechanism of MIH1 gene inactivation in the MSI sporadic cases. We propose the inclusion of this method in the diagnostics of CRC, as a step following the analysis for MSI and prior to the screening for mutations in the MMR genes.

Immunohistochemistry. MMR mutation screening. Genotype - phenotype correlations

The IHC showed lack of MLH1 expression in all detected tumors with methylation. Patients whose tumors showed hMLH1 promoter hypermethylation were not included in the screening for MMR mutations. Our search for genetic alterations in hMIH1 and hMSH2 genes found the following variations: four known and two novel mutations, one unpublished and four known intronic polymorphisms in both hMLH1 and hMSH2 genes.

PCR amplification of all excns including flanking intronic regions was performed and than analyzed using single-strand conformation polymorphism (SSCP) analysis. Samples showing an altered mobility pattern were then subjected to direct sequencing.

Our search for genetic alterations in hMIH1 and hMSH2 genes found the following variations: four known and two novel mutations, one unpublished and four known intronic polymorphisms in both hMLH1 and hMSH2 genes. Lack of MLH1 protein expression was shown in all tumors with previously described pathogenic mutations as well as in the tumor from the novel frameshift mutation.

We aimed to analyze the segregation of the novel mutation within the pedigree and correlated the observed clinical features to this molecular defect on both genomic and protein levels. Four tumors were analyzed for microsatellite instability (MSI) and for MIH1 protein expression using the immunohistochemical (IHC) assay. In the three adenocarcinomas MSI and lack of the MIH1 protein expression were detected. The only one tubulovilous adenoma analyzed was microsatellite stable (MSS) and the MIH1 protein showed an intact staining.

The clinical evaluation of the cases revealed typical features of HNPCC such as early age of

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cancer development, dominant inheritance and anticipation. The colorectal cancers were predominantly right-sided, with extracellular mucinous production, presence of synchronous and metachronous tumors. A specific pathologic manifestation was the high differentiation of the colon adenocarcinomas. Beside the extracolonic tumors involved in the HNPCC spectrum, one of the family members developed a benign giant cell soft tissue tumor of the third finger, after primary colon localization. We conclude that the mutation c.31delC is HNPCC causative, with high penetration and a prophylactic program for all mutation carriers has been proposed.

Association studies of the role of polymorphisms in candidate genes

Different epidemiological studies report protective effect on colorectal cancer exerted by vitamin  $D_3$  intake, estrogen replacement therapy and increase of the risk of MSI in CRC by the estrogens withdrawal.

In 2004 the Science Fund of Sofia Medical University financed one-year research project "Association study of the role of polymorphisms in Vitamin D and Estrogen Receptor type alpha genes in patients with colorectal cancer".

The aim of our study was to search for association between CRC and polymorphisms in Estrogen Receptor (ER) and Vitamin D receptor (VDR) genes. We analyzed the PvuII and XbaI polymorphisms from the ER gene and the BsmI polymorphism of the VDR gene in 140 patients with CRC (subsequently divided according to their MSI status) and 94 controls. We concluded that PvuII and XbaI polymorphisms in the ER- $\alpha$  gene were associated with the risk of developing MSI in CRC patients. The BsmI polymorphism in the VDR gene was linked to the risk of developing CRC.

In brief, during the last seven years we developed an efficient diagnostic strategy for HNPCC testing and the mutation status of 80% MSI HNPCC cases that fulfill the Amsterdam criteria could be detected. In the whole group, 28% were proved as sporadic cases. The PCR-based HpaII method for detection of the MLH1 promoter region hypermethylation has been proved as a simple and applicable method in the diagnostic algorithm for CRC. We propose this method in the diagnostics of CRC as a step that follows the analysis for MSI and prior to the screening for mutations in the MMR genes, especially in sporadic cases, because in most cases they are mutation negative. The use of IHC analysis improves the detection rate and might be a very helpful tool in the investigation of some unclear molecular variations.

### Team members:

Colorectal Cancer

Kaneva R., PhD; Kremensky I., MD, PhD - Laboratory of Molecular Pathology, University Hospital of Obstetrics and Gynaecology, Sofia

Nedin D., MD, PhD; Alexandrova A., MD; Kostadinov E., MD; Tzancheva M., MD; Damyanov D., MD, PhD - Clinic of Abdominal Surgery, Queen Giovanna Hospital, Sofia,

**Gegova A., MD, PhD -** Department of Pathology, Queen Giovanna Hospital, Sofia,

Hristova T., MD - Department of Histology, Medical University, Sofia,

Mitev V., MD, DSc - Department of Chemistry and Biochemistry, Medical University, Sofia,

Vlaykova T., PhD; Galabova M., PhD -Department of Chemistry and Biochemistry, Trakyan University, St. Zagora

### ENDOMETRIAL CANCER

Endometrial cancer (EC) is the most common gynecologic malignancy. The mean age to develop EC is approximately 63 years and most of the cases are sporadic. However, a subset of women (5%) with EC have features suggestive of an underlying inherited susceptibility to cancer. Endometrial carcinoma is the second most common malignancy in the cancer syndrome, hereditary non-polyposis colon cancer (HNPCC).

The HNPCC syndrome is associated with a defect in DNA mismatch repair. In HNPCC kindreds the most common cancers are colorectal and endometrial. HNPCC is most frequently associated with MLH1 and MSH2 mutations. Mutations in the MSH6 mismatch repair gene may account for a specific risk for EC. Loss of DNA mismatch repair in cancer cells leads to microsatellite instability (MSI). MSI in endometrial cancers without family history are associated with MLH1 promoter hypermethylation.

In 2005 a three-year research project "Molecular diagnosis of familial endometrial cancer in Bulgaria" was funded by the National Science Fund. This project aims to study the molecular mechanisms of developing familial EC in Bulgaria.

Clinical and biological material from 150 patients with EC at the age under 50 years will be collected. These patients will be genetically characterized by analyses for MSI, promoter hypermethylation of the MLH1 gene, immunohistochemistry and screening for mutations in the main MMR genes. The optimal strategy for early diagnosis and molecular diagnostics of EC will be proposed. The project will initiate a familial EC register. Information brochures about possibilities for early detection and prophylactics of the EC will be prepared for patients and specialists.

The project brings together the research potential of a multidisciplinary team of gynaecological surgeons, pathologists and molecular biologists from leading medical universities, hospitals and a NGO for the fulfillment of the project aims.

### Team members: Endometrial Cancer

Kadiyska T., PhD; Kaneva R., PhD; Kremensky I., MD, PhD - Laboratory of Molecular Pathology, University Hospital of Obstetrics and Gynaecology, Sofia,

Ivanov St., MD, PhD - Clinic of Gynaecology, National Oncological Institute, Sofia, Tiufekchieva E., MD, PhD; Dimitrov R., MD, PhD; Mainkhard K., MD; Doganov N., MD, - University Hospital of Obstetrics and Gynaecology, Sofia,

Vlaykova T., PhD; Galabova M., PhD -Department of Chemistry and Biochemistry, Trakyan University, St. Zagora.

### BIOCHEMICAL DIAGNOSIS OF LYSOSMAL STORAGE DISEASES IN BULGARIA

Ivanka Sinigerska, MSc; Irfet Hassanova; Katerina Vladimirova, Assoc. Prof. Ivo Kremensky, MD, PhD Laboratory of Molecular Pathology, Sofia

Lysosomal storage disorders (LSD) are a group of more than 40 different genetic diseases. They occur in total in about 1 in 7000-8000 live births and display considerable clinical and biochemical heterogeneity. The common feature of these diseases is the defective activity of a lysosomal protein (result of a single gene mutation) that is part of a catabolic pathway. This prevents the further breakdown of complex compounds and leads to an intralysosomal accumulation of partially degraded material. Most often the deficient protein is a specific enzyme, responsible for one step in the sequential degradation of sphingolipids, glycoproteins, mucopolysaccharides and glycogen. Other causes of LSD are the deficiency of the so-called activator protein assisting a lysosomal enzyme in its catabolic function (in GM2-AB variant), or the deficiency of a component of larger lysosomal enzyme complex (SAP deficiencies). Inappropriate lysosomal enzyme targeting (in mucolipidoses) and defective export of degradation products from lysosomes (in sialic acid storage disease) also can lead to LSD . The resulting clinical symptoms develop progressively, ranging from coarse facial features, bone changes and organomegaly, to neurological problems and psychomotor retardation. LSD can be classified depending on the pathway affected and the nature of accumulated substrate - mucopolisaccharidoses, glyaproteinoses, lipidoses.

A precise diagnosis is needed as early as possible to allow adequate treatment, therapy and genetic counseling. At present there is no effective therapy for the most severe LSD, leading to premature death. Enzyme replacement therapy is limited to three diseases (Gaucher, Fabry, and MPS I) and is quite expensive. The majority of LSD is inherited in an autosomal recessive manner (except Xlinked Hunter, Fabry and Danone diseases) and the high 25 % recurrent risk in subsequent pregnancies refrain many families from having further children. The prenatal diagnosis (providing selective termination of affected fetus is acceptable) is an action of choice only in cases with previously strongly confirmed enzymological or molecular diagnosis of the genetic defect.

The initial approach to the diagnosis of LSD is based on clinical features. The well-known symptoms might be highly suggestive, but the correct diagnosis of the genetic defect is often difficult because of the overlapping phenotypes and quite often is complicated by the fact that most of the diseases are quite rare and moreover clinically heterogeneous. Clinicopathological findings and identification of abnormally stored substances may reinforce the clinical suspicion but they are not diagnostic. There is frequent overlap of storage material between different diseases. Analysis of urinary metabolites facilitates differential diagnosis of ISD, pointing to the probable enzyme defect. Enzymatic diagnosis offers the chance of a definitive result and also makes possible prenatal diagnosis in future prequancies in the family.

The enzymatic diagnosis of LSD in Bulgaria was introduced 30 years ago by Prof. I. Kremensky, Prof. L. Kalaydjieva and Dr. R. Varon. Till now, with the great help and support of Enzyme laboratory, Department of Clinical Genetics - Rotterdam, the Netherlands, the number of assays has been expanded and the applied procedures improved. At present 24 enzyme tests, listed in Table 1, are performed in the Laboratory of Molecular Pathology. In Bulgaria the postnatal and prenatal diagnosis of 26 LSD, resulting from enzyme deficiency (from altogether 30 known), is possible.

It is not practical to examine all known enzyme deficits in every particular case. Appropriate scheme of assays should be performed, depending on the observed key features. A screening flowchart for biochemical diagnosis of LSD was developed in our laboratory. The goal of our work was to detect rapidly and reliably most of the known LSD and to offer prenatal diagnosis for them.

More than 3000 patients, aged from several days up to 52 years, suspected of having LSD, from the whole country, as well as patients from Macedonia and Serbia, have been referred to the laboratory for biochemical diagnosis.

Laboratory diagnosis of mucopolysaccharidoses is generally achieved by three sequential steps: quantitative detection of excessive excretion of glycosaminoglicans (CAGs) in urine followed by qualitative analysis to determine the probable MPS type and finally demonstration of the specific enzyme defect in plasma, leukocytes or cultured skin fibroblasts. The GAGs were measured using own modification of 1, 9-dimethylmethylene blue (DMB) method. Age-dependant reference limits were determined using the program REFVAL, recommended by the International Federation of Clinical Chemistry. From 1996 to 2001 an external quality assessment scheme for accuracy control, provided by the European Research Network for evaluation and improvement of screening, diagnosis and treatment of Inherited Disorders of Metabolism (ERNDIM) was carried out. The results obtained were within one standard deviation, calculated from values reported by all 72 European laboratories participating in the scheme.

Fractionation of GAGs has been performed by thin layer chromatography (TLC) or one-dimensional electrophoresis. Enzyme assays have been carried out using syntetic fluorogenic substrates (4-methylumbelliferone derivatives). The patients' range of enzyme activity has been less than 10 % from the control group range.

Thin layer chromatography of oligosaccharides has been applied as a first step in biochemical diagnosis of glycoproteinoses, followed by confirmatory enzyme assays in the cases with abnormal pattern.

For diagnosis of lipidoses enzyme tests relevant to clinical symptoms have been performed.

Determination of plasma levels of chitotriosidase has been used as a screening test.

This non-lysosomal enzyme, originating from activated macrophages, has been markedly elevated in patients with Gaucher disease and in some other LSD (Niemann-Pick, Krabbe, Pompe, Wolman) in a less extent. Using the flowchart and the methods already mentioned for more than 3000 patients, suspected of having LSD, the correct diagnosis has been obtained on a total of 189 patients. In 56 cases diagnosis has been set on metabolite level only, due to the lack of material for enzyme assay. The diagnostic rate about 8.2 % is rather close to the value given by Prof. David Wenger, Jefferson Medical College, Philadelphia (about 8.0 %) and has to be regarded as confirmation of the usefulness of our diagnostic algorithm. Mucopo-lysaccharidoses (90 cases) are the most common disorders of diagnosed LSD in our laboratory - 38.5%. Significant number of patients with Niemann-Pick (29 patients), all from gypsy origin, as well as 27 cases of GM1, 26 cases of MLD, 26 cases of Krabe have been detected. We do not pretend that the confirmed LSD diagnoses correspond to the real prevalence of these disorders in Bulgarian population. For 25-year period prenatal diagnosis has been attempted in 41 cases (13 of them with the cooperation of Dr.W. Kleijer, Department of Clinical Genetics, Rotterdam). The enzyme activities have been measured in cultured amniotic fluid cells (40 cases), cell-free amniotic fluid (2 cases) and cord blood leukocytes (1 case). The results from enzyme assay in cell-free amniotic fluid have been confirmed by enzyme assays in cultured amniocytes. In 11 cases of prenatal diagnosis (26.8% from all) the results have indicated affected fetuses and pregnancies had to be terminated. The diagnoses have been confirmed when fetal tissues were available.

### Table 1. List of LSD, corresponding enzyme assayed, number of diagnosed patients and prenatal diagnoses in the Laboratory of molecular pathology.

	DISEASE	ENZYME	Patients	Prenatal Diagnoses
1	MPS I Hurler/Scheie	α-Iduranidase	3	
2	MPS II Hunter	Iduronate 2-sulfatase	14	6
3	MPS III A Sanfilippo A	Heparan Sulphamidase	7	
4	MPSIII B Sanfilippo B	N-acetyl $\alpha$ - glucosaminidase	7	2
5	MPSIII C Sanfilippo C	Acetyl-CoA: α-glucosaminide N-acetyltranspherase		
6	MPS IV A Morquio A	N-acetylgalactosamine 6-sulphatase	3	1
7	MPSIV B Morquio B	$\beta$ -Galactosidase	1	
8	MPSVI Maroteaux-Lamy	Arylsulphatase B	8	1
9	MPS VII Sly	β-Glucuronidase		
10	Mucosulphatidosis	ArylsulphataseA , ArylsulphataseB	2	
1	Pompe disease	$\alpha$ -Glucosidase	5	1
12	Aspartylglucosaminuria	Aspartylglucosaminidase		
13	Fucosidosis	α-Fucosidase	1	3
14	a-Mannosidosis	$\alpha$ -Mannosidase	1	
15	b-Mannosidosis	$\beta$ -Mannosidase		
16	Schindler disease	$\alpha$ -N-acetylgalactosaminidase		
17	Fabry disease	$\alpha$ -Galactosidase	3	
18	Gaucher disease	β-Glucosidase	26	3
19	GMI-ganglicsidosis	β-Galactosidase	27	12
20	GM2-gangliosidosis (Sandhoff)	Hexosaminidase A+B	4	1
21	GM2-ganglicsidosis(Tay-Sachs)	HexosaminidaseA	2	3
22	Krabbe disease	Galactocerebrosidase	12	3
23	Metachromatic leukodistrophy	Arylsulphatase A	26	3
24	Nieman -Pick disease A &B	Sphyngomielinase	29	1
25	Wolman disease	Acid lipase	1	
26	Mcolipidosis I (Sialidosis)	Neuraminidase	1	
27	Mucolipidosis II (I cell disease)	Multiple lysosomal hydrolases	6	1
	Total		189	41

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### PRENATAL DIAGNOSIS OF THE MOST COMMON HUMAN ANEUPLOIDIES WITH DNA ANALYSIS

 Silvia Andonova; Radostina Raynova, MSc; Assoc. Prof. Ivo Kremensky, MD, PhD - Laboratory of Molecular Pathology, University Hospital of Obstetrics and Gynaecology, Sofia Medical University and genetic councellers from the Department of Medical Genetics and Department of Paediatrics, Sofia Medical University, Tel: + 359-2-9172473, Fax: +359-2-9172469; E-mail: sandonova@netscape.net
 Violeta Dimitrova; Valentina Mazneykova - University Hospital of Obstetrics and Gynaecology, Sofia

Prenatal cytogenetic analysis in high risk pregnancies for chromosome abnormality has been performed routinely since the early 1970s. Despite of the improved cell culturing and chromosome banding, it takes around 4 weeks from the collection of the sample till the reporting of the results from conventional cytogenetic analysis. The long lasting procedure have been recognized as main disadvantage of this analysis and was one of the main reasons for the introduction of new molecular methods for prenatal diagnosis of common autosomal aneupoidies.

With the development of DNA technologies a new diagnostic method has been proposed since the 1990s - indirect quantitative fluorescent PCR analysis (QF-PCR). It is based on the investigation of highly polymorphic DNA markers (short tandem repeats - STRs), located on the target chromosomes. The use of STR markers with a high heterozygosity will decrease the probability of uninformative results. The addition of any supplemental polymorphic marker per chromosome would increase the diagnostic accuracy in most if not all investigated samples. The high throughput, low cost and accuracy of this technique made it a reliable method of choice for detection of the most common human aneuploidies - trisomies 21, 18 and 13 (causing Down, Edwards and Patau syndrome, respectively), as well as triploidy. QF-PCR method has been successfully applied for rapid prenatal diagnosis of aneuploidy by a number of UK and European labs.

Since the introduction of QF-PCR analysis in Bulgaria (2000) more than 1300 fetuses were diagnosed. Investigated pregnancies ranged from 10 to 34 weeks of gestation (most of them between 16 and 20 week). Pregnant women were

referred for prenatal diagnosis because of one or more indications: increased maternal age (most frequent indication), previous child or a pregnancy with a chromosomal abnormality or multiple malformations, elevated risk from Down syndrome second trimester serum screening, abnormal ultrasound scan, or parental anxiety. Most of the samples were obtained after invasive prenatal procedure - usually anniccentesis or chorionic villus sampling. Genomic DNA was extracted from different fetal tissues - anniotic fluid samples, chorionic villi, cultivated anniotic cells, fetal tissue samples after abortions, or fetal blood cells collected after cordocentesis. Most of the QF-PCR results were verified by a conventional cytogenetic analysis in a parallel study. DNA samples with structural chromosomal abnormalities on other chromosomes, not included in our analysis will not be detected with QF-PCR analysis. The method allows detection of maternal contamination by the presence of additional alleles or by comparison with the genotype of the mother, when possible.

A total of 4 STR markers for chromosome 21 (D21S11, D21S1411, D21S1440, D21S1435), 3 - for chromosome 18 (D18S535, D18S51, D18S858) and 3 - for chromosome 13 (D13S631, D13S258, D13S256) in four multiplex PCR assays were used routinely for QF-PCR analysis in Bulgaria. These markers were selected because of their high heterozygosity reported in literature. Detection of the sex chromosomes numerical disorders and particularly Turner syndrome is in progress.

Thirty of the fetus samples were diagnosed as trisomic for chromosome 21, 8 - for chromosome 18, 3 - for chromosome 13 and 1 sample was diagnosed with triploidy with at least 2 STR markers (see figure 1).

#### Conclusions

QF-PCR technique is a reliable and rapid alternative for prenatal diagnosis of trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome) and trisomy 13 (Patau syndrome), especially when some problems with cell cultivation arise and/or cytogenetic analysis is impossible. QF-PCR analysis could be an option in prenatal and postnatal diagnosis in cases with strong suspicion for particular autosomal aneuploidies (including chromosomes 21, 18, 13) especially in small countries with restricted resources like Bulgaria. However, because a limited number of chromosomes are involved in this study, the classical cytogenetic analysis cannot be fully replaced at this stage of our work. Our results indicate that DNA markers we use are highly informative for our population and are suitable for the purposes of prenatal diagnosis in Bulgaria.

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Figure 1: Electrophoretogram showing the QF-PCR detection of a 47,XY,+21 fetus. M - Mother, F- Father



### BULGARIAN ADDED VALUE TO ERA

### THE STEPHAN ANGELOFF INSTITUTE OF MICROBIOLOGY - CENTER OF BULGARIAN MICROBIOLOGICAL SCIENCE

Prof. Maria Angelova, DSc; Assoc. Prof. Lyubka Doumanova, PhD; Prof. Angel Galabov,DSc, Corr. Mem. The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 26, Acad. G. Bonchev Str., 1113 Sofia, Tel. +359 2 8701081, Fax +359 2 8700109, E-mail: lillyh@microbio.bas.bg

The Institute of Microbiology was founded on March 7, 1947 by the world-famous Bulgarian scientist Prof. Stephan Angeloff, Member of the Bulgarian Academy of Sciences (BAS), who became its first director for 15 years. Stephan Angeloff graduated from the Berlin University in 1901 as a veterinary doctor. He specialized in microbiology in the laboratory of Robert Koch in Berlin and was a student of his most close collaborators: Paul Ehrlich, Friedrich Loffler, and August von Wasserman. Stephan Angeloff was the scientist, who on the eve of the First World War, using own original vaccine, built up a barrier on the way of the rinderpest into the European continent. He was the founder of the Bulgarian microbiology, the creator of a solid scientific school in all directions of microbiology.

The establishment of the Institute of Microbiology realized the necessity of formation of a research center with national importance for carrying out contemporary investigations in rapidly developing and prosperous branches of microbiological science. Five units were founded at first in the Institute: bacteriological (medical, agricultural, and industrial bacteriology), protozoological, virological (investigation of viruses and viral diseases in man, animals, and plants), immunological, and biochemical.

During the initial period of its development (1947-1962) the Institute was connected with studies of the current after the Second World War infectious pathology of domestic animals and humans under the direct leadership of Prof. S. Angeloff and his first collaborator Prof. Ilia Kujumdjiev. The achievements in the study of the etiology and the development of diagnostic methods and immunoprophylactic preparations for these infections were significant. Bacteria, mycoplasma and L forms, antibiosis and antibacterial medicinals, as well as agents causing protozoic invasions were also studied. The beginning of the investigations in the field of infectious immunology was set by Prof. Vladimir Markov, Member of BAS. Industrial microbiology started with investigations of Prof. Ignat Emanuilov, Member of BAS, on the microbiology of milk and dairy products, biochemistry and physiology of lactic acid bacteria and application of bacterial enzymes (proteinases) in cheese making. Alongside with bacteria as producers of biologically active substances, filamentous fungi were studied. About 1964 the investigators were grouped into two principal fields: I. Metabolism and biosynthesis of microorganisms and their regulation; II. Nature and regularities of the interactions between microorganisms and the macroorganism.

In the field of microbial morphology electron microscopic studies of bacteria, filamentous fungi, yeasts and host immune system cells were performed since 1964. The investigators in the field of microbial biochemistry after 1973 aimed at elucidation at a contemporary biochemical level of the main peculiarities in the physiology and biosynthetic . . . . . . . . . . . . . . . . . .

capabilities of microbial cells. The main object of these studies were some enzymes (ribonucleases, acid and alkaline phosphatases from yeasts, bacterial neuraminidase and luciferase); proteinase and nuclease inhibitors; microbiological transformation of steroids; protein-lipid interactions within the bacterial membrane in regard to the secretion of extracellular enzymes. As a result of systemic investigations carried out by Prof. Simeon Galabov and his collaborators, the relationship between antigenic structure and biological properties of bacterial endotoxins were clarified by the use of different methods for their detoxication. Data were obtained concerning medical application of endotoxins, based on their antigenicity (diagnostic kits), imunogenicity (vaccines), interferon-inducing activity, and tumor-necrotic action.

The research work in the field of microbial genetics was developed by Prof. Kalcho Markov, Corresponding Member of BAS. The investigations were connected with the problem of prophage interference, genetic transfer in *Bacillus thuringiensis* and *Streptococci* of group A, hybridization of *Actinanyces sp.* in order to increase their productivity, construction of gene maps of *Streptomycetes* – producers of antibiotics, molecular identification of microorganisms by the use of PCR, as well as genomic differentiation by ML3 DNA fingerprinting and ribotyping.

The Institute of Microbiology has always played a leading role in applied microbiology. Microbial producers of a wide spectrum of biologically active substances were isolated: enzymes, amino acids, proteins, carctinoids, and various antibiotics with wide spectrum of activity. The physiology and biochemistry of microbial producers, including extremphilic bacteria (themophilic and alkalophilic), as well as properties of the synthesized products were studied in detail. A wide spectrum of methods for immobilization of enzymes and microbial cells-producers of biologically active substances, were elaborated.

The investigations in infectious bacteriology after 1962 were pointed on the changes provoked in the pathogen during the course of infectious and immunizing process (Prof. Alexander Toshkov, Corresponding Member of BAS, and collaborators). Later research was focused on the factors and mechanisms of bacterial virulence and bacteria - host interactions using as a model Yersinia infection (Prof. Dimitar Veljanov and collaborators).

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At the beginning of the 60s investigations in the field of experimental chemotherapy of viral infections were started. The search for viral inhibitors from Bulgarian medicinal plants (tested mainly on influenza viruses) was extended later to systemic studies on the creation and characterization of some new effective antivirals (vs. influenza, enteroviruses, flaviviruses and togaviruses), as well as on the combined effects of viral inhibitors (efficient against herpes- and picomaviruses) and their mechanisms of action. Studies on the etiology and pathogenesis of some viral infections were recently started.

After the pioneer in Bulgarian immunology works carried out by Prof. V. Markov, the investigations on cell immunity and L forms of bacteria under the guidance of Prof. Valchan Valchanov and Prof. Assen Toshkov followed. A wide spectrum immunopharmacological screening was performed aiming at the selection of new active immunomodulatory substances.

In 2004 the Stephan Angeloff Institute of Microbiology was admitted as an associated member of the Pasteur Institute in Paris - the sixth associated member in Europe. This is a great recognition for the Institute and for the Bulgarian Academy of Sciences by this world centre of microbiology, which is the leader in the fight against contagious diseases. The association to Pasteur Institute family provides an opportunity for enlargement of collaboration between Bulgarian and French microbiologists, and microbiologists from other European Institutes belonging to Pasteur Institute network (Lille, Brussels, Sankt-Peterbourg, Bucharest, Athens, and Rome).

At present the staff of the Institute of Microbiology consists of 171 persons, including 86 scientific researchers (35 professors and associate professors). The main directions of the scientific research in the Institute are:

#### Bacteriology

• Factors and mechanisms of bacterial virulence - lipopolysaccharide, membrane proteins, enzymes, etc.

• Pathogenesis of yersiniosis, lysteriosis, melliobsis, thularemia, etc.

• Genetically constructed bacterial vectors for elaborating a new generation of vaccines classical swine plaque, etc.

• Testing of different natural and synthetic products for antibacterial activity.

• Bacterial resistance to antimicrobial agents.

• Vaginal lactobacilli - molecular - genetic analysis and their role in the protection against sexually transferred infections.

• Bacteriocins.

• Elaborating methods for microbiological control of food products

#### Virology

• Experimental chemotherapy of viral infections.

• Development of effective anti-adenoviral chemotherapy.

• Antiviral substances vs. toga- and flavivings.

• New virus inhibitors from synthetic and natural origin (plant, microbial, etc.).

• Biological response modifiers (BRM) immunandulators, interferon inducers, antioxidants, etc.

• Combined effects of antiviral substances and BRM.

• Antiviral microbicides - screening system, development of new generation of viral disinfectants.

• Mechanisms of action of viral inhibitors.

• Role of some viruses in human pathology (Balkan endemic nephropathy, diabetes, cervical cancer).

#### Infectious immunology

• Immunopharmacological investigation on substances with natural origin (microbial, plant) and synthetic products.

• Studying on their immunomodulating effect in experimental animal models of infections and of tumors.

• Autoimmune disorders resulting from infection.

• Formulation of the conception for physiological autoimmunity.

• Mechanisms of control of autoimmunity.

• Investigation of the role of natural autoantibodies in the autoimune diseases.

• Reestablishing the tolerance to DNA with gene-engineering chimeric molecules - a new

approach for treatment of systemic lupus erythematosus.

#### Applied microbiology

• Genetic analysis of microorganisms, used in the food and pharmaceutical industries - lactobacilli, streptonycetes, etc.

• Characterization of the microbial cell response to physiological stress.

• Selecting and characterizing of microorganisms, used as starter cultures in dairy production.

• Production, characterization and application range of biologically active microbial products:

- enzymes incl. thermo stable - superoxide disutase, pullularase, inulinase, protesses, pectinases, anylases, TAQ- polymerase, neuraminidase, etc.

- organic acids, vitamins and growth factors.

- amino acids.
- -biosurfactants.
- -antibiotics.

• Microbiological methods for steroid transformation.

• Cell cultures of endemic plants - a source of biologically active substances for the food and pharmaceutical industries.

• Mathematical modeling of biotechnological processes.

• Microbial ecology:

-microbiological monitoring of soils and water.

- the role of microorganisms and surfactants in bioremediation and biodegradation.

- technology for biogas production.

### Public health

• Virulence factors in bacteria-causative agents of socially important zoonosis (optimizing of diagnostics, therapy, and immunoprophylaxis).

• Introduction of molecular methods for early diagnostics of tuberculosis in children and adults.

• New methods for detection of intestinal infections and toxic infections in foods of animal origin.

• Epidemiological investigation on the role of human papilloma viruses in cervical cancer.

• Balkan endemic nephropathy - etiopathogenesis, epidemiology, immune status.

• Immunoglobulin preparations for treatment of autoimmune diseases.

• Chemotherapy of epidemic adenoviral keratoonjunctivitis.

• Monitoring of disinfectants as main components in hospital hygiene for control of nosocomial infections.

• Participation in the national strategy in the control of infectious diseases - diagnostics, epidemiology, vaccines.

• Participation in the Commission for Eradication of Policnyelitis at the Ministry of Public Health as part of the WHO program.

• Participation in Biological Safety Expert Council at the Permanent Commission for Prevention of the Population from Natural Disastres and Catastrophes.

• Participation in Research and Development Expert Group on Countering the Effects of Biological and Chemical Terrorism, European Union.

• Informing by mass media on the current for the country problems of infectious diseases.

### Education activity

The Stephan Angeloff Institute of Microbiology is authorised and certified by the Highest Testimonial Committee and the Ministry of Education and Science as a national institution for training and preparing of graduated students in obtaining a doctor degree in three fields: microbiology, virology and immunology.

Three International Training Courses for young scientists were organized at the Atelie of the Institut Pasteur at the Stephan Angeloff Institute of Microbiology:

1 Molecular methods for diagnostic and taxonomic identification of bacteria and fungi (23 October - 8 November 2002).

2 Molecular methods for diagnostic and taxonomic identification of viruses and fungi (6 - 14 November 2003).

3 Methods for DNA finger-printing and genotyping of drug-resistant Mycobacterium tuberculosis strains" (25-30 October, 2004).

The courses aimed to provide training in molecular biology methods for identification and typing of pathogens. High level standing lectures on molecular biology of medically important microorganisms as well as seminars dedicated to the recent breakthroughs in the field and practical training on DNA extraction, PCR, RT-PCR, and nonradioactive hybridization were included in the frame of scientific program. Eminent scientists from Pasteur Institutes - Paris (France), Sankt-Peterburg (Russia), Cantacuzino (Romania) and The Stephan Angeloff Institute of Microbiology Sofia (Bulgaria) were invited to deliver lectures and practical exercises. Young scientists from Bulgaria, Greece, R. Macedonia, and Romania attended the courses. They were highly estimated both by the participants and the invited lecturers. The courses provided opportunity for future scientific collaboration, exchange of information and friendship. A collaboration project "Macrophage dependent immunoprotective action of Candida albicans DNA" (the project coordinator Assoc. Prof. N. Ivanovska) was granted by ACIP program in September 2005. Institute Pasteur Paris, France supported five fellowships for Bulgarian microbiologists.

The education activity includes also participation in Leonardo da Vinchi Programme of EC. Our scientists created innovative programmes for vocational training in healthcare microbiology and immunology as well as in production of healthy sound bioproducts for improvement of skills and competence of the staff.

The Professors, Assoc. Professors and some Junior Scientific Workers from the Institute deliver lectures and provide experimental exercises to the master degree students in many universities all over the country (as well as abroad) in more than 20 subjects - Sofia University St. Kliment Chridski, Medical University - Sofia, University of Forestry - Sofia, Prof. Dr. A. Zlatarov University - Bourgas, Technical University of Sofia, Technical University - Gabrovo, Technical University - Dresden, South-West University - Blagoevgrad, University - Bitola, etc. Many of the students received their master degree after making the thesis work in different laboratories of the Institute. For the previous 2004-year the lectures and exercises were 1233.

Great number of scientific workers from different departments consults many institutions (govemmental, university, private, etc.) on problems of ecology and environment, public health, agriculture and food industry. Some members of the Institute are involved in the work of many expert groups, for example, Research and Development Expert Group on Countering the Effects of Biological and Chemical Terrorism at EC, Biological Safety Expert

Council at the Permanent Commission for Prevention of the Population from Natural Disasters and Catastrophes, Expert Council for Epidemiological Control of Infectious Diseases, Immunoprophylaxis and Commission for Eradication of Policyelitis at the Ministry of Public Health, National Agencies of Standardization and Authorization, Scientific Councils of different institutions, etc. The Institute of Microbiology is the Head Office of the Balkan Society of Microbiology, Bulgarian Society of Microbiology, as well as a Center for organizing national and international congresses, conferences and workshops in the field of microbiology.

The Director of the Institute is a member of the Expert Advisory Board, the Council of International Union of Microbiological Societies, Council of Federation of European Microbiological Societies, and Council of Directors of Institute Pasteur and associated Institutes. He participates in the work of FEMS, IUMS, WHO, International Society for Antiviral Research, etc.

During the recent years the aim of our research activity was to maintain a high level of scientific programs with the modern directions in biosciences that are in close relation with the national priorities. The projects developed in the Institute of Microbiology are financially supported by the National Fund for Scientific Investigations (Ministry of Education and Science), other ministries, foundations, and Bulgarian companies (48) as well as by the European Union, NATO, UNESCO, and other international organizations and companies (40).

Among our most important achievements of research activity we count the following: Intervention strategies against the development of drug-resistant tuberculosis.

There is an alarming increase of tuberculosis incidence in Bulgaria: from 25.1 per 100000 population in 1990 to 50.1 in 1998 and 48 in 2001, as well as of MDR-TB incidence (to 8.6% of the total registered TB case load). International collaboration of the Department of Pathogenic Bacteria with German scientific institutions focuses on a recent development in tuberculosis research, which is the increasing recognition of the importance of drug-resistant populations of *Mycobacterium tuberculosis* in patients. Present investigations contribute to understanding of the nature of "heteroresistance" as a prerequisite for development of MDR-TB and will fundamentally benefit patients by more effective chemotherapy. The researchers have the unique opportunities to get acquainted with advanced technologies and international cooperation, thus contributing to improvement of MDR-TB case detection rate in Bulgaria.

Yersinia enterocolitica is an important causative agent of intestinal lesions including acute enteritis, enterocolitis and mesenterical lymphadenitis.

In rare cases, particularly in immuno-compromised hosts, Y. enterocolitica can induce systemic infections with abscesses in spleen, liver and other organs. Our investigations focus on the roles of different virulence factors of Y. enterocolitica required for pig colonization and persistence. We studied the pathogenicity and immunogenicity of wild-type and different lipopolysaccharide mutant strains of Y. enterocolitica serotype 0:8 and Y. pseudotuberculosis, serotype 0:3, using a pig oral model of infection. We followed in vivo the colonization of different organs and the histopathological and immunomorphological changes associated with the infection. Also the ability of the mutant strains to resist different host defence mechanisms of pigs was studied. We were able to select the mutant strains, which are highly promising and applicable for further construction of live carrier vaccine.

Discovery and characterization of new antiviral substances - inhibitors of viral replication products of chemical synthesis or of natural origin.

> 1-(4) -Morpholinomethyl-1(1Í) tetrahydropyrimidinone (mopyridone) is characterized as a potent inhibitor of replication of influenza viruses A (H2N2, H3N2) and B, and of togaviruses (species of genera *Alphavirus* and *Rubivirus* - rubella virus) as well. A high efficacy of mopyridone was proved in experimental influenza A (H3N2) and infections in albino mice. The compound manifests high activity in experimental alphavirus infections in mice. On the base of its protective effect in infections with massive virus inocula and high values of selectivity index mopyridone could be considered as the most active anti-alphavirus substance described in literature.

> Aporphinoid alkaloid oxoglaucin (isolated and synthesized in the Center of Phytochemistry, Institute of Organic Chemistry, BAS) possesses strong and broad-spectrum anti-enterovirus activity *in vitro* and a marked effect in experimental Cox-sackie B1 neuroinfection in newborn mice.

> Polyphenol-rich extract from the medicinal plant Geranium sanguineum L. merits special attention among the series of other substances of plant origin showing antiviral activity in our laboratory. The extract showed a broad spectrum of antimicrobial activities; in addition to inhibiting the replication of influenza viruses type A and B in vitro and in ovo, the preparation suppressed the growth of a series of pathogenic bacteria and fungi. The anti-influenza virus effect of the preparation in vitro was shown to be specific and selective and was directed against an early stage of infection. The antiviral effect can be attributed to the presence of a variety of biologically active compounds as well as the possible synergistic interactions between them. The application of the extract in the course of the influenza virus infection in mice lead to a notable immunorestorating effect and a significant improvement of the antioxidant defense system. Thus, the significant protection in vivo might be attributed to a combination of multiple biological activities.

Determination of the molecular mechanisms of antiviral action of antivirals - ligands of viral proteins discovered in our laboratory and the chemical structure - antiviral activity relationship.

> Clearing-up of the mechanism of action of mopyridone on influenza virus replication. It was found that mopyridone is capable to form a stable complex with influenza virus structural protein VP1, thus counteracting the interaction of M1 protein with the lipid bilayer membranes. Study on mopyridone-resistant and mopyridone-sensitive mutants of influenza virus A (H3N2) established significant differences between them in the physicochemical characteristics of virions, the antigene structure (sights 1A, 2 and 3) and the aminoacid content of M1 protein, as well as in the capability of M1 bonding in liposomes. In sum, these data characterized M1 protein as the target of anti-influenza virus effect of mopyridone.

Establishment of the chemical structure
 antiviral activity relationship in cyclic ureas and

thicureas and on this base realization of directed synthesis of active derivatives.

Study of the drug-resistance phenomenon in enterovirus capsid-binding inhibitors, a barrier for the development of highly efficient antiviral chemotherapy.

> It was established that disoxaril- resistant enterovirus population was developed in the brain of laboratory animals experimentally infected with Coxsackie B1 virus and treated with disoxaril (one of the blockers pocket of the hydrophobic capsid protein VP1 - the most effective enterovirus inhibitors). This explains the mild therapeutic effect of the compound observed in vivo.

 $\succ$  Disoxaril - dependent enterovirus mutant was developed.

> Significant differences in the phenotypic draacteristics of the disoxaril Coxsackievinus BI mutants were established. The resistant mutants are with less thermostability, larger size (S+) and more irregular shape of virus plaques under agar overlay and manifest slightly increased pathogenicity formice. A genetic markers' complex for drug mutants is developed as a requirement for the research on anti-enteroviral chemotherapeutic agents.

> It was proved that lack of disoxaril for the disoxaril-dependent mutant blocks the process of assembly of infectious virions and exerts no effect during uncoating. This finding is in contrast to the effect of disoxaril on the disoxarilsensitive virus population (characterized by binding to the target VP1).

### Study of the combination effects of picornavirus replication inhibitors.

> The synergistic inhibitory effect of dual combinations of disoxaril, arildone, S-7, PTU-23, HBB and enviroxime was proved against the replication of some enteroviruses (Coxsackie B1 viruses, poliovirus type 1, ECHO13 virus). A promising chemotherapeutic prospect of the combinations (lack of cross-resistance and lack of synergistic cytotoxicity) and a marked antiviral effect in vivo were established.

> The triazole nucleoside ribavirin proved to be an antagonist of all tested enteroviral inhibitors when applied in combination with them. > In order to restrict the process of development of drug resistance, an original scheme for combined administration of enteroviral inhibitors in vivo was developed.

> Establishment of a markedly synergistic combination effect of the most effective anti-flu chemotherapeutic agents rimantadine-hydrochloride and oseltamivir in experimental infection with influenza virus A/HBN2 in albino mice.

Proven several effective synergistic combinations vs. herpes simplex viruses 1 and 2: ribavirin + acyclovir, methotrexate + BVDU, mizoribine + acyclovir, etc.

> Establishment of a synergistic combination effect of idouridine and sidofovir on vaccinia virus replication *in vitro*.

### Establishment of antiviral effect of cycluridine vs. Flaviviruses and its efficacy in the treatment of the flavivirus infections in cattle (mucosal diseases).

> Establishment of anti-flaviviral activity of cycluridine (a cyclis urea synthesized in Bulgarian laboratory): inhibitory effect on flavivirus replication on the model of the bovine pestivirus (BVDV), etiological agent of the mucosal diseases in calves, as well as a marked protective effect in experimental BVDV infection in calves.

> Establishment of protective effect of cycluridine in lethal experimental infection with the flavivinus of tick-born encephalitis in albinomice. This study was carried out in collaboration with Prof. I. F. Barinsky et al. (D. I. Ivanovskii Institute of Virology, Russian Academy of Medical Sciences, Moscow). Results obtained are unique with the proved efficacy of this chemotherapeutic agent in this especially dangerous infection with a high lethality in humans.

> Establishment of a marked chemotherapeutic effect of cycluridine in natural infection of mucosal diseases - viral diarrhea in calves. Development of optimal treatment course of oral administration (doses, duration, etc.).

> Cycluridine is the first efficacious chemotherapeutic agent described in literature vs. this infection damaging cattle-breeding in this country and on a global scale), and one of the first active agents vs. flavivinus infections at all occupying an important place in the infectious pathology in humans (hepatitis C included).

### Oxidative stress in the pathogenesis of flu and antioxidants.

> Characterization of the role of oxidative damage ("oxidative stress") in the flu pathogenesis (in albino mice infected with influenza virus A/H3N2). Tracing out the markers of oxidative stress - lipid peroxidation products, anti-oxidant enzyme activities, liver monooxigenase enzyme, endogenous antioxidant levels - as vessels-damaging factors.

> Proving the protective action of antioxidants of natural (vitamins E and C) and synthetic origin [methyl-2,6-ditretbutylphenol (Yonol)] on the course of influenza virus infection (with influenza virus A/H3N2 in albino mice) by tracing the changes in oxidative stress markers.

> Establishment of the fact that ant iflu chemotherapeutic agent rimantadine-hydrochloride significantly decreases the oxidative stress markers' values during the course of influenza virus infection. The compound does not possess antioxidant properties (does not influence induction of both lipid peroxidation and antioxidant enzymes in various model systems, does not interact with superoxide). Its antioxidant-like action is based on its inhibitory effect on influenza virus replication.

> Cold and/or immobilization stress, preceding the influenza virus (A/HBN2) infection, causes a drastic increase of oxidative damages in the body (in liver, lungs, blood plasma, stomach) and as a consequence a severe course of the flu pathogenesis.

### Zinc-containing complexes in influenza virus infection.

> Establishment of protective effect of zinccontaining complexes (zinc diglycinate and other compounds) in experimental infection with influenza virus A/HBN2 in albino mice.

### Characterization of a new-type virucidal agent for hand disinfection.

> Determination of the antiviral spectrum of the highly effective and broad-spectrum virucidal composition, a new-type hand disinfectant, Manorapid<sup>®</sup> Synergy, introduced in industrial production and in the clinical practice (USA, Canada, Germany, Bulgaria).

> Large-spectrum system for *in vitro* screening of virucidal agents: Development and introduction of a large-spectrum system for *in vitro* screening of virucidal agents.

### Studies on the Balkan endemic nephropathy (BEN).

> Role of viruses in etiology and pathogenesis of BEN: Data are obtained about the role of hantavirus infection in BEN as a result of seroepidemiological investigation of BEN patients and by electron microscopy of surgery materials.

> Role of the immune system in BEN etiopathogenesis: Characterization of the immune status in BEN patients by phenotyping of lymphocytes and determination of phagocytic activity of macrophages by flowcytometry.

> Determination of neopterin level (in urine) in BEN patients and its correlation with other basic chlinico-laboratory markers.

### Studies on the problem viruses - diabetes.

> Diabetogenic action of herpesviruses: Replication and damaging action of a representative of herpesviruses selectively in Langerhans islets' B cells was established for the first time (Aujesky disease virus, pseudorabies virus on the model of organ culture of Langerhans islets from pigs).

> Concept for diabetogenic action of neurotropic viruses: Concept for diabetogenic action of neurotropic viruses is formulated, based on the theory of the paraneuronal nature of pancreatic endocrimecells.

### Papillomaviruses in Bulgarian population.

Molecular epidemiological study by PCR on the role of human papillomaviruses as cancerogens (precancerous states and cervical cancer) on Bulgarian population was realized for the first time.

### Autoreactivity of therapeutic immunoglobulin preparations.

The binding to human liver antigens of a panel of seven different connercial IVIg preparations was compared. The results indicated that the binding of IgG molecules from IVIg to self-antigens correlated to the treatment at low pH during the production. The treatment of IVIg at low pH resulted in increasing of their inhibitory effect on proliferation of human peripheral blood cells. The effects of different IVIg preparations on an experimental sepsis in mice were also studied. IVIgs with high and low selfbinding may have different immunomodulating impacts when infused to autoimmune patients.

### Mechanism of action of plant substances in experimental tumors.

Significant evidence was obtained on the effect of substances of plant origin in experimental tumors in mice. The mechanisms of their action on the antitumor effector cells and on the release of reactive oxygen and nitrogen species and cytokines, participating in the antitumor defense are revealed. The obtained results are important for the complete characterization of these plant metabolites as substances stimulating the immune response in malignant diseases.

### Selective suppression by a chimeric antibody of DNA-specific B cells in SLE.

A hybrid antibody molecule was constructed that is expected to down-regulate specifically the proliferation and differentiation of dsDNA-binding B cells from lupus patients. This engineered molecule containing a monoclonal antibody against the human complement receptor 1 (CR1, CD35) coupled to a peptide that mimics antigenically DNA is expected to target selectively B-cells recognizing native DNA and to deliver to them a strong suppressive signal via their inhibitory surface CR1 receptors. We have produced chimeric antibodies by coupling a monoclonal anti-human CR1 antibody to the peptide DWEYSVWLSN that mimics the antigenic epitopes of dsDNA. The number of IqG anti-dsDNA antibodies producing cells was reduced after treated PEMCs with chimeric antibodies contain monoclonal antibody against the human complement receptor 1 (CR1, CD35) coupled to a peptide that mimics antigenically DNA. We show in this study that it is possible to suppress selectively autoreactive B lymphocytes by using an antibody chimera contain monoclonal antibody against the human complement receptor 1 (CR1, CD35) coupled to a peptide that mimics antigenically DNA.

### Reactivity of human natural IgG autoantibodies to serum proteins is directed mainly to circulating immunoglobulins.

Natural polyreactive IgG autoantibodies are known to be present in the plasma of diseasefree individuals and as a result - in pooled human therapeutic intravenous immunoglobulin G (IVIg) preparations. Early studies have shown that that serum proteins are one of the groups of selfantigens to which natural autoantibodies bind. We analyzed the spectrum of serum proteins, bound by F(ab)' fragments of IVIg. The human serum was passed through a column with immobilized  $F(ab)'_{a}$  fragments of IVIg or a column with  $F(ab)'_{a}$ fragments of a human immune monoclonal IgG antibody (as a negative control). The spectra of proteins in the eluates from the both affinity columns were analyzed by 2D electrophoresis and by blotting. The results indicate that a therapeutic IgG, obtained from a large group of healthy plasma donors binds to circulating immunoglobulin molecules and to an additional group of serum proteins. The blotting experiments confirmed that IqA, IgG and IgM are bound by the immobilized  $F(ab)'_{2}$ fragments of IVIg. This result comes as no surprise as it has been shown before that idiotype/ antiidiotype interactions take place between immunoglobulin molecules in immunoglobulin preparations. The affinity of the binding of natural autoantibodies to self-antigens is known to be low, with K values in the micromolar range. Whether these interactions take place in vivo and whether they have biological significance obviously depends on the concentration of both ligands. As both the concentrations of serum IgG, IgA and IgM, and the kD values of idiotype/antiidiotype interactions between them are in the micromolar range, this binding should take place also under physiological conditions in vivo. Very large doses (30 and more grams) of pooled IgG are administered by the intravenous route to autoimmune patients and thus these interactions may also be relevant to the immunomodulatory activity of the IVIg treatment.

### Identification of lactic acid bacteria from different ecological niches and characterization of antimicrobial peptides (bacteriocins) produced.

> In order to assess the important role of vaginal lactobacilli in maintaining the vaginal health and in the prevention or therapy of bacterial vaginosis a molecular-genetic study of Bulgarian vaginal lactobacilli was carried out. Twenty-two active strains were identified and grouped according to their genetic relatedness, based on a combined approach which included amplified ribosomal DNA restriction analysis (ARDRA), ribotyping and polymerase chain reaction (PCR) with species-specific oligonucleotide primers. All vaginal isolates and reference stains were grouped in 3 clusters in comparison to a set of 21 reference strains based on the initial ARDRA results, which was confirmed by ribotyping. Finally, the strains were subjected to PCR analysis with 7 different species-specific primers, which allowed most of them to be classified as belonging to one of the following species: Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus crispatus, Lactobacillus plantarum, and Lactobacillus gasseri. This is the first molecular-genetic study of vaqinal lactobacilli isolated from Bulgarian women, as the predominant species was L. fermentum.

> The cryptic plasmid pt38 (2911 bp) of Streptococcus thermophilus 2783, a strain isolated from Bulgarian yogurt, was subcloned and sequenced. Five ORFs were identified. ORF2 would specify a 142-amino acid protein sharing a high degree of homology with plasmid-born low-molecular-weight heat stress proteins described in a variety of S. thermophilus strains. The plasmid pt38replicates via a rolling circle mechanism A DNA region that can be transcribed into a small RNA (ctRNA) complementary to the leader segment of the rep (ORF1) mRNA is proposed to be involved in the control of plasmid replication. In vitro synthesis of this ctRNA was observed, and this constitutes the first report on the existence of such antisense RNAs, likely acting as regulatory elements, in S. thermophilus plasmids.

Physiology and resources of lactic acid bacteria and health benefit solutions. To exploit the biological potential of lactic acid bacteria for making original Bulgarian starter cultures for wholesome dairy products of high nutritive value we studied the unique natural biosystems that defined the species diversity of lactic acid microflora, and identified localities of biotypes of lactic acid bacteria. The investigations positioned strains of lactobacteria according to their ability to synthesize biologically active substances – providers of health benefits, as well as according to the levels of proteolytic enzymes. The main achievement related to creating multi-component synbiotic starters by incorporating highly productive strains of lactobacteria

into strains with well-proven technological characteristics, i.e. through multistrain natural simulation. Microbial cell response against environmental stress. The efforts were focused on the characterization of cell response against different types of stress. Low multicellular eukaryotes such as filamentous fungi were used as a model. The main finding is that the oxidative stress is involved in the phenomena of heavy metal stress, heat- and coldshock. The results strongly suggest causal link between stress degree and enhanced levels of free radicals; increased content of oxidative damaged intracellular proteins; accelerated synthesis of reserve carbohydrates and changes in activity of antioxidant enzyme defence. New information about the role of oxidative stress in fungal cell aging was dotained.

### Role of dephosphorylating enzymes in cell physiology and metabolism.

> For the first time it was shown that the specific pho 13 alkaline phosphatase (4-nitrophenyl phosphate alkaline phosphatase) from Saccharomices cerevisiae possessed high dephosphorylating activity towards phosphorylated protein substrates. The newly demonstrated property may provide a clue to elucidate the unclear role of this enzyme in the yeast cells.

> Yeast protein phosphoseryl phosphatase (PSPP) with phosphorylase phosphatase action in contrast to the described so far PSPP was found to be non-dependent on metal ions, thus resembling the protein phosphatases Type 1 in high eukaryotes.

### Investigation on extremophilic bacteria.

Search for novel and biotechnologically unexplored microorganisms as well as striving for better understanding of biodiversity motivates our interest to the habitants of the extreme niches.

> Four new bacterial strains representing unknown phylogenetic units were isolated from Bulgarian hot springs. Two of them are suggested as new species from new genus, *Calorbacillus rupii* and *Anylobacillus thermophilus*. These results are the beginning of microflora investigations in Bulgarian hot springs and contribute to the world knowledge of biodiversity on our planet.

> After screening procedure from samples of polluted industrial waters a moderate thermophilic bacterial strain *Bacillus sp.* UG-5B with benznitrile degrading activity was isolated. The strain is a high producer of thermostable nitrilase with very broad substrate spectrum, hydrolyzing aliphatic, carbocyclic and heterocyclic nitriles. Effective immobilized systems for enzyme production were carried out. These biocatalysts show increased termostability and perspective for different applications (for chemical synthesis and detoxification processes).

> Many of the strains originally isolated from Bulgarian hot springs are producers of thermostable enzymes of great biotechnological importance. One of those strains is the only producer of thermostable gellan lyase reported up to now. The enzyme has an application in many branches of food industry. Its thermostability determines the possibility for running industrial processes at temperature higher than 60°C at which temperature polysaccharides (including gellan) solubility is much higher.

> From samples (flowing spring water, water with soil particles, alga bacterial mud and mud) collected near different thermal Bulgarian springs, 20 bacterial strains with superoxide dismutase activity (SOD) were isolated. The relatively high SOD activity (1300 U/ml) and thermostability (the half enzyme life was 30 min at 90°C) of *Bacillus* sp. isolate M 20 characterized it as a perspective producer of this antioxidant enzyme.

### Investigations on Antarctic microorganisms.

A collection of psychrophilic, psychrotrophic and mesophilic yeasts and filamentous fungi, isolated from Antarctic samples provided by the Bulgarian Antarctic Expeditions at the Livingston Island was made. The biological potential and metabolic ability of selected Antarctic mycetes were studied. The active producers synthesing polysaccharides, enzymes and lipids were taxonomically classified. The conditions for biosynthesis and physicochemical characterization of glucomannan were established. The cultivation conditions and properties of b-glucosidase were explored. Lipid composition of Antarctic yeasts with low lipid and unsaturated fatty acid content was estimated.

Microbial transformation of the steroid compounds sitosterol and androstenedione was performed by whole resting cells of *Mycobacterium*  sp. NRRL B-3805 and *Rhodococcus* sp., respectively, in a single phase organic solvent based medium. In both cases cells retained their viability and revealed high steroid transforming activity. The investigations were performed in collaboration with Centro de Engenharia Biológica e Quimica, Instituto Superior Técnico, Lisboa, Portugal.

Surface-active compounds.

It was found that nonionic detergents increased the permeability of yeast cells for low molecular substrates. On this base a simple, rapid, and mild procedure allowing determining periplasmic enzyme activities in whole microbial cells was proposed. Recently the interest in microbial surfactants increased due to their diversity and environmentally friendly nature. The effect of rhamolipid biosurfactants, produced by Pseudomonas strains on microbial growth, cell-surface properties (hydrophobicity and permeability), composition and fine structures of cell membrane and wall of bacterial and yeast strains and bacterial monocultures isolated from polluted sites were studied. The biochemical and ultrastructural studies showed that the biosurfactants affected the surface structures of different microbes in different degree, leading to different changes in cell abilities. These results will elucidate the role of rhannolipid-biosurfactants on the biodegradation of low soluble compounds and will extend the theoretical foundations for the development of efficient technologies for bioremediation of polluted sites.

The microbial utilization of aromatic compounds represents a potential solution to environmental protection problems.

The available scientific literature data concerning catabolic genes in yeast species, in particular genes coding for enzymes of the phenol degrading pathway is quite insufficient. The methods for discovering new catabolic genes, as well as genes involved in the bioaugmentation process of the environment demonstrate the advantages of unique and easily identified molecular markers for the investigation of natural microbial populations. The aim of our work is to contribute to more exact and complete information about the diversity and specificity of genes codyng the enzymes degrading aromatics. The investigated *Trichosporon cutaneum* R57 yeast strain possesses an exclusive degradation effectivity compared to other reported microbial phenol degrading agents which was confirmed by the biochemical analyses of the first three enzymes included in phenol degradation pathway. The sequence analysis of the specific phenol hydroxilase, catechol dioxygenase and cis, cis-muconate cyclase genes leads to new possibilities for a specific response to the growing environmental pollution with toxic aromatic compounds.

Anaerobic digestion of organic wastes is a biotechnology which mineralizes different in origin organic wastes and as a result compost and biogas (energy source) are produced. The increased interest of many European countries in this biotechnology is due to the solving of energy and environmental problems. On the basis of the research in laboratory "pseudohomogeneous" bioreactors with organic wastes from farming, food industry and activated sludge and different mixtures of these wastes in different proportions we formulated the following main conclusions:

> For specific mixtures of organic waste biogas production could increase significantly and at the same time the stability of the process is maintained;

> The use of appropriate stimulating substances and surface-active compounds could also increase biogas production, but it is necessary to do techno-economic calculations in order to take efficient decisions;

> Anaerobic digestion of organic wastes in a single "pseudohomogeneous" biogas reactor does not significantly decrease the COD of the effluent compared to the influent, i.e. the degree of depollution is not enough. In a "cascade" of two bioreactors with different working volumes it is possible to increase significantly the depollution effect;

> The community of different groups of microorganisms in the biogas reactor is sometimes influenced by some hard-to-measure environmental factors (such as geomagnetic activity and atmosphere pressure) even at stable physicochemical variables (ex. t °C and pH in the biogas reactor), which is one of the reasons leading to unexpected "failures" of the process.

A modified chemostat technique called titrostat was developed for selective isolation and continuous flow cultivation of rapidly growing bacteria utilizing toluene vapours as a single source of carbon and energy. In this technique the toluene was fed via the air as an independent flow and all other components entered the cultivation space according to the protocol of pH-control module. The system operates at very low residual toluene concentrations, which along with the high mass transfer properties of the homogeneous cultivation allows the system to be regarded as a possible device for cleaning the air from volatile organic compounds.

### Biological active substances from plant *in vitro* cultures.

Bioactive compounds currently extracted from plants are used as food additives, pigments, dyes, insecticides, cosmetics and perfumes and fine chemicals. However, decreased plant resources together with environmental problems have prompted industries, as well as scientists, to consider the possibilities of investigating in vitro systems as an alternative supply for the production of plant secondary metabolites.

> Laboratory technology for the production of rosmarinic acid by L. vera MM was developed. After investigation of the relationships in the biological system "Nutrient medium – Lavandula vera MM cell culture – rosmarinic acid" and the next optimization of the nutrient medium and conditions of the bioreactor cultivation of L. vera MM cell suspension, maximum yield of 3484.4 mg/L rosmarinic acid was achieved.

> The technology for the transfer of the Ri plasmid from A. *rhizogenes* to the plant cells was successfully applied for the red beet. It was established that biosynthesized betalains from obtained hairy root cultures possess high radical scavenging activity and they were stable at the *in vitro* conditions of the gastrointestinal tract.

> Ten original two-phase systems were developed on the base of polyethylene glycol and dextran. The phase characteristics and the distribution of the nutrients and cells between phases were defined. For the fist time investigated aqueous two-phase systems were used as a medium for cultivation of plant cell suspensions.

> Alkaloid spectra of Datura stramonium, Datura inoxia, Atropa belladonna hairy root cultures, Leucojum aestivum callus and shoot cultures, as well as volatile and polar compounds in Rosa damascena Mill 1803 cell suspension were investigated. For the first time it was proved that *D. stramonium* hairy root culture biosynthesized 3tigloyloxy-6-propionyloxy-7-hydroxytropane.

Many results obtained in scientific investigations are prepared to be applied in practice. The most significant are the following:

### GLUCOMACROPEPTIDE FROM MILK WHEY

**Area of application:** In laboratory practice; enzymology; human and veterinary medicine

Annotation: A laboratory technology for obtaining of glucomacropeptide from milk whey has been developed. The product is with molecular weight 8000D. It is used as a substrate for determining neuraminidase activity of viruses and microbes. The glucomacropeptide also shows features of an imunostimulator to humoral immune response in humans and animals.

Readiness stage: Laboratory technology.

### DETERMINATION OF BONDED AND FREE SIALIC ACIDS

Area of application: In laboratory practice; human and veterinary medicine.

**Annotation:** Methods for determination of bonded and free sialic acids in glycoproteins, glycolipids and oligosaccharides are used.

Readiness stage: Laboratory analyses.

### ISOLATION AND CHARACTERIZATION OF LAC-TIC BACTERIA

**Area of application:** Lactic bacteria, as potential starters for food industry, animal breeding and medicine.

Annotation: We offer methodical approaches and protocols for characterization of Lactic bacteria as well as determination of their probiotic potential,

for development of microbial food additives with (useful) health effect, applied in food industry. We offer identification of perspective strains of Lactic bacteria, which is an acquirement when these strains are used as starters. We offer also pasportization of starters (for yogurt production, cheese production, etc.) for their patent protection.

Readiness stage: Methods for laboratory expertise.

### MICROBIAL FOOD ANALYSIS

**Area of application:** Food industry, animal breeding and medicine.
**Annotation:** We offer analysis to determine undesired microflora in foods, using up to date methods, as well as to prove microorganisms to be useful and to prove their vitality.

Readiness stage: Laboratory expertise.

## STUDY OF SUBSTANCES FOR ANTIMICROBIAL ACTIVITY AND FOR THEIR BIO-PROTECTIVE EFFECT

Area of application: Pharmaceutical industry. Annotation: We offer screening investigations of substances possessing antimicrobial activity to prove their potential and application as bio-protectors. Readiness stage: Laboratory expertise, consultations.

ELECTRON-MICROSCOPIC EXPERTISE (SCAN-NING AND TRANSMISSION ELECTRON MICROS-COPY) FOR MORPHOLOGICAL CHARACTERIZA-TION OF WHOLE CELLS AND ULTRATHIN SEC-TIONS OF VARIOUS MICROORGANISMS

Area of application: biological monitoring, medicine, biotechnology, microbial diagnostics and taxonomy.

Annotation: A variety of electron microscopy protocols have been approbated and modified for specific application to different microbial taxa. The appropriate choice of protocols allows both ultrastructural characterization of microorganism species and strains and also their changes under the impact of environmental factors. This can be applied for expert evaluation of cellular microbehost interactions or of microorganism changes in the course of biotechnological processes.

**Readiness stage:** Laboratory expertise and scientific consultations.

MICROBIAL TECHNOLOGY FOR PRODUCTION OF NOVEL ANTIOXIDANT ENZYME, Cu/Zn-CONTEINING SUPEROXIDE DISMUTASE (HLSOD) Area of application: medicine, pharmaceutical and cosmetic industry, enzyme production industry.

Annotation: A highly efficient microbial technology for production of purified enzyme preparation Cu/Zn-SOD has been elaborated. The novel antioxidant enzyme is naturally glycosylated and can be isolated in very few cases only. Such SOD exerts a far higher pharmacological activity than non-glycosylated enzymes. HLSOD showed a protective effect against myeloid Graffi tumour, in experimental influenza virus infection, and cryoconservation of farm-animal sperm. This enzyme may be used in:

> Oncology (protection against tumour appearance; retardation of tumour progression; reduction of cytotoxic and cardiotoxic effects of antitumour drugs);

Cryoconservation of cells and organs;

> Transplantation of organs.

**Readiness stage:** Laboratory technology, knowhow, and consultations.

## METHODS FOR THE DETECTION OF DEGRA-DA-TIVE FUNGI

**Area of application:** museums, libraries, leatherenterprises, shops, stores.

Annotation: Methods for detection and identification of filamentous fungi included in biodegradation of wide range of polymens (wood, leather, textile, historical records, etc.) have been developed.

**Readiness stage:** Laboratory expertise, consultations, instructions for preservation and elimination of fungi.

# METHODS FOR THE DETECTION, ISOLATION AND CHARACTERIZATION OF FOOD-BORN FUNGI

Area of application: food industry enterprises (bakery, confectionery, sausage etc.)

Annotation: Methods for detection and identification of food-born fungi have been developed. Readiness stage: Laboratory expertise, consultations, instructions for preservation and elimination of fungi.

## ONE-STEP HYDROLYSIS OF STARCH TO GLUCOSE Area of glucose syrup application:

>food industry: confectionary, production of ice creams, soft drinks, baby foods, dressings;

 $\succ$  ethanol production;

> yeast protein for stock-breeding;

> raw material for production of glucosefructose syrups. They represent much more healthy sweetener than sucrose and intensively replace it in confectionary.

**Amotation:** Laboratory technology includes simultaneous action of four thermostable enzymes ( $\alpha$ -amylase,  $\beta$ -amylase, pullulanase and glucoamylase) with similar pH and temperature optima instead of traditionally used 3-stage process. The hydrolysis is per-

formed at 60°C, temperature at which starch is soluble. Readiness of technology: laboratory technology.

## DETERMINATION OF PHARMACOLOGICAL AC-TIVITY OF BIO-SUBSTANCES

## Area of application: Pharmaceutical industry, food industry, agriculture, etc.

Annotation: Via group of lab methods we test different substances possessing bioactivity as newly obtained drugs, as food additives and fodder additives for animals; newly synthesized chemical compounds, etc. We study the effect of these substances on different model experimental animals, on their immune systems, as well as on test microorganisms, as follows: pyrogen activity (in vitro and in vivo); toxicity (in vivo); influence on complement activity; functional activity of phagocyte cells (phagocyte test, killing test, oxygen and nitrite radicals production); cytokine induction (interleukins, tumor necrotizing factor, interferon, etc.); antibacterial activity; artifingal activity.

**Readiness stage:** Established methods for lab expertise of the above-mentioned items.

## DETERMINATION OF COLI-TITER, TOTAL NUMBER UNITS (CFU)

Area of application: Sanitary control of plant departments, conveyors, raw materials, etc., used in food industry for drinks and foods production, etc, as well as the products themselves.

Annotation: Assessment and control of total microbial contamination is of great importance for quality and stability (durability) of the entire food, pharmaceutical, cosmetic, etc. products. Effective internal department control could be reached, using proposed methods.

**Readiness stage:** Established methods for lab assessment of hygiene and quality of the corresponding (relevant) products.

## METHODS FOR INCREASING OF ANTIBIOTIC AC-TIVITY OF INDUSTRIAL STRAINS-PRODUCERS OF ANTIBIOTICS

**Area of application:** Pharmacy industry – for production of antibiotics for use in human and veterinary medicine.

Annotation: Wide range of methods (from induced mutagenesis to modern molecular-genetical techniques) are applied for increasing the activity of industrial strains-producers of important antibiotics used in human and veterinary medicine. Readiness stage: Laboratory technology.

## BIOTECHNOLOGICAL PROCESSES FOR REMOVAL OF HEAVY METALS FROM WASTE WATER AND INDUSTRIAL SOLUTIONS

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Area of application: The object of the biotechnological processes is connected with removal and concentration of copper, cobalt, iron and other heavy metals from waste, industrial or mining water and following regeneration of the biosorbent resulting to concentration and extraction of the metals in solutions.

**Annotation:** The biotechnological process is characterized by repeated use of sorbent (fungal biomass), possibility for extraction of desirable metal by electrolize and high economic efficiency in comparison to classical chemical methods.

**Readiness stage:** Laboratory technology with possibility forgetting patents.

## ROSMARINIC ACID PRODUCTION BY LAVAN-DULA VERA MM PLANT CELL SUSPENSION

Area of application: Food, cosmetic and pharmacertical industries.

Arnotation: Rosmarinic acid possesses various biological activities (antimicrobial, anti-inflammatory, antimutagenic) and also shows strong radical scavenging activities. These features reveal possibilities for its application in food, pharmaceutical and cosmetic industries.

**Readiness stage:** Laboratory technology and scaleup to 3L bioreactor.

## BETALAINS BIOSYNTHESIS BY *BETA VULGARIS* L. HAIRY ROOT CULTURE

Area of application: Food, cosmetic and pharmacertical industries.

Annotation: The betalaines are water-soluble natural colorants, including the red-violet betacyanins and the yellow-orange betaxanthines. They possess strong radical scavenging activity and are stable in pH and temperature area of the most food technologies, which defines them as appropriate colorants in foods, cosmetics and pharmaceuticals.

Readiness stage: Laboratory technology.

## BIOSYNTHESIS, PROPERTIES AND BIOLOGICAL ACTION OF EXOMANAN FROM YEASTS

**Area of application:** Stock breeding, plant protection, medicine.

Annotation: The proposed technology is for ob-

taining polysaccharide manan, which, when added to animal feed, inhibits intestinal colonization by pathogens. In vivo trials with animals show positive results - decreased mortality rates, increase of productivity and improved health status, which defines the polymer as a new biologically active substance for application in stockbreeding. The biological action of manan was proved through its inhibitory effect on phytopathogens, establishing its role as ecologically friendly means for plant protection. For its antimicrobial effect towards clinical staphylococcal strains, manan can also be considered as an antiinfection means for human application.

**Readiness stage:** Laboratory technology, tested at semi-industrial scale.

## BIODEGRADATION OF TOXIC AROMATIC COM-POUNDS IN WASTE WATERS

Area of application: elaboration or optimization of technologies for detoxification of wastewaters and soils, contaminated in the result of oil processing and organic synthesis productions.

Annotation: A yeast strain, with high effectiveness in phenol and its toxic derivatives degradation was selected and characterized. It was proved, that the strain is capable of degrading other toxic compounds, components of the wastewaters of some chemical productions, such as: metilstyren, acetone, benzoic acid, methanol. For the first time it was established that, unlike other biodegrading agents, this strain is capable of degrading highly toxic compounds isopropylbenzene and dimethylcarbinole. The cells of the strain maintain their high activity after covalent immobilization on polyacrilamide carriers. **Readiness stage:** Laboratory technology, consultations.

## CREATION AND INTRODUCTION OF THE ANTI-VIRAL CHEMOTHERAPEUTIC ÀDENOSTATIN COLLYRIUM<sup>®</sup>

**Area of application:** medicine, antiviral chemotherapy.

Annotation: The antiviral chemotherapeutic Àdenostatin Collyrium<sup>®</sup> for treatment of ocular viral infections with adenoviral etiology (epidemic keratoconjunctivitis, etc.) was elaborated. It is the most efficacious anti-adenoviral chemotherapeutic agent and the first Bulgarian antiviral chemotherapeutic. The preparative dossier is prepared in view of the interest of leading pharmaceutical companies from USA and Japan.

**Readiness stage:** Introduced for industrial production and in clinical ophthalmologic practice.

#### MARITSA VEGETABLE CROPS RESEARCH INSTITUTE - 75 YEARS OF SERVICE

Dr Stoyka Masheva; Dr Miroslav Michov; Mr Galina Pevicharova; Dr. Dimitrina Kostova

Maritsa Vegetable Crops Research Institute 32, Brezovsko shosse, 4003 Plovdiv, Bulgaria Phone number: +359 32 952296, Fax number: +359 32 960177

Email: izk@plov.omega.bg

Maritsa Institute, founded in 1930, has developed over the years as a leading center for vegetable research in Bulgaria. Combining scientific studies with practical application, the Institute has yielded remarkable successes in different fields including the development of vegetable hybrids, improvement and stabilizing of local forms and accessions to high-yielded and high-quality cultivars, and amelioration of vegetable growing technologies.

Prominent researchers from Maritsa Institute have created close links to agricultural universities and educational institutions to transfer modern horticultural methods and knowledge. Looking forward to Bulgaria membership in the European Union, Maritsa Institute has developed fruitful collaborations with related institutions in member countries.

The Institute will join the European Research Area focusing on transferring European knowledge and technology and to further develop the Maritsa Institute as a leader and promoter of Vegetable Crops Improvement for Bulgaria and the Balkans.

#### INTRODUCTION

#### Historical Background

Bulgaria is a country with a long tradition in vegetable growing, due to favorable climatic conditions, as well as great diversity of vegetable forms and accessions with unique and desirable properties. For centuries Bulgarian gardeners were well known in Europe, especially in the Danube region of the Austrian Empire. Even today, fine restaurants in Vienna buy their fresh vegetables from the Bulgarian vegetable market. Together with other agricultural branches, vegetable growing made considerable progress in Bulgaria at the end of the 19th century, enriching local traditions with modern methods. However it was realized that advances in modern agriculture could not be achieved without science. As a result an Experimental Agricultural Station, mainly devoted to vegetables, was established in Plovdiv on April 1, 1930. The nucleus of the Station was built on the ground of a large private farm with excellent fertile soil. Later the Institute was named the Maritsa Vegetable Crops Research Institute, after the famous Maritsa river, the ancient Hebros, which rises from the Rila Mountains, transverses the town of Plovdiv, and irrigates the province of Tracia, until it reaches the Aegean Sea. The first successes came soon. In 1932 Professor Hristo Daskalov and his research team carried out profound theoretical investigations on heterosis and released the first F, tomato hybrids the same year. Due to its excellent quality, this hybrid remained in production for more than 30 years. Several years later heterosis breeding was applied successfully to eggplant, pepper, and cucumber. The Institute also played a significant role in elaborating the theoretical basis for hybrid seed production and its application into practice. At that time Bulgaria became a world leader in yield and total production of hybrid seeds. In 1950, total production was 10,000 - 12,000 kg of tomato hybrid seeds, with yields of 200 - 250 kg/ha. During this early stage, laboratories for quality control and technological analysis were established, striving to evaluate unique local germplasm collected from all over the country and preserved in the Institute.

During the period of 75 years more than 300 cultivars and F, hybrids were created and widely spread throughout the country. Some of them, because of their valuable characteristics, were exploited in foreign breeding programs. Maritsa Institute also became famous as a pioneer of new technologies in vegetable production. The unique and traditional Bulgarian method for vegetable growing on "phytarion" (small areas enclosed with berms), was mechanized and adapted for bean, pea and later for processing tomato, red pepper, and potato. The Institute was also a pioneer in greenhouse vegetable production and constructed the first greenhouses for tomato and cucumber. Furthermore, the Institute developed the first plant tissue culture laboratory for potato and flower crops, established an international station for potato breeding and cultivar maintenance with German participation, elaborated technology for biological pest control, and utilized hydroponics systems for vegetable growing.

For years the Institute has been a coordinator of scientific research from several local experimental stations established on different climatic zones and soil types: in Negovan (on flowers and leaf vegetables), in Samokov and in Smolyan (on potato), in Gorna Oryahovitsa (on onion and cucumber). The Agricultural University of Plovdiv invites many leading scientists from Maritsa Institute as prominent lecturers and teachers of new generations of horticulturists and agronomists in the fields of genetics, breeding, and agrotechnology. Research groups of the Institute are involved in international collaboration with corresponding institutions in the Netherlands, Italy, France, England, Spain, Germany, Hungary, Poland, Czech Republic, countries of the former Yugoslavia, Russia, and Moldova.

#### Structure of the Institute

Maritsa Vegetable Crop Research Institute is a legal entity in Bulgaria, and a Member of the National Center for Agrarian Science (NCAS) under the Ministry of Agriculture and Forestry. It comprises two main Departments: (1) Breeding, Variety Maintenance and Introduction and (2) Technology-for-Growing. The people playing major roles in the Breeding Department are the breeders of the most important Bulgarian vegetables including tomato, pepper, cabbage, cucumber, potato, onion and garlic, bean and pea. Their research is supported by investigations carried out in seven laboratories that are included as separate units in the departments having long experience in different aspects of vegetable technology: Vegetable Fruit Quality, Virus Immunity, Plant Tissue Culture, Cytogenetics, Molecular Investigation, Physiology, and Vegetable Processing).

For 75 years the Breeding Department has been a coordinator and executor in the creation and implementation of a large number of cultivars and F, hybrids of the principal vegetable crops of Bulgaria. A great diversity of local and introduced forms and cultivars, breeding lines and populations of wild species has been developed. Most of the local cultivars (Bulgaria is a secondary centre of origin for pepper, head cabbage, bean, and onion) have been stabilized and improved through the breeding programme of the Institute, distinguished by high nutritional value and organoleptic characteristics that make them competitive on the market. The Department works with a rich gene pool from these crops, variable in their morphological and biological characters. There are 14 PhD scientific officers, including 6 senior members. In addition, there are 6 professors as associate members. The scientific staff of the Department has a long-standing experience in vegetable breeding, and has acquired considerable practice in managing projects supported by the Bulgarian Ministry of Science and Education, and Ministry of Agriculture and Forestry. An international project on red pepper quality, developed by INCO-COPERNICUS 94, was successfully completed with participation of the pepper breeding team and Vegetable Fruit Quality Laboratory. There is an ongoing project, funded by the International Atomic Energy Agency (IAEA) in Vienna, on the induction and study of tomato and pepper mutants with high nutritive quality. The Breeding Department is also responsible for cultivar maintenance and seed preservation (gene bank).

The Technology-for-Growing Department comprises the following units: Agrotechnology, Plant Protection, Plant Nutrition, and Mechanization. The scientific staff includes 10 PhD officers, 6 of whom are senior, and an additional 3 professors as associate members. Great emphasis on investigations in Plant Protection Unit is now being given to development of sustainable control systems and crop protection strategies. The team of Plant Nutrition directs its efforts towards investigations on bioproducts as alternative solutions to improve nutrient regime and plant growth. The team of Agrotechnology deals with the influence of the main environment factors on yield. Current research on mechanized technologies concerns analysis on input-output energy for growing vegetables in open and protected fields, in order to decrease the energy ratio. There is an on-going project on improvement of greenhouse crops production technology and efficiency, funded by FAO [TCP/BUL/ 3002 (A)]. The Institute is also involved in organization of training and extension activities in the Vegetable Sector, part of the joint project, entitled "Strengthening of Fresh Fruit and Vegetable Marketing Quality Standards Control System" (PPA/ 04/BG/9/1), funded by the Netherlands Ministry of Economy. A significant part of Maritsa research programs integrates the efforts of scientists from different structural units of the Institute.

#### RESEARCH TOPICS

## The priority investigations of the Institute comprise a number of topics as follows:

> Enhancement of vegetable quality by improving biological value (increasing the components with antioxidant effect), sensory characteristics, pest and diseases resistance, high temperature and drought tolerance by the use of conventional and biotechnological breeding methods;

> Evaluation and exploitation of various vegetable gemplasms, comprising local and introduced accessions, breeding lines, and cultivars;

>Improvement of integrating systems for pest control (pest, diseases and weeds) in vegetables;

> Investigations on bio-products as alternative solutions to improve nutrient regime, plant growth and plant qualit;

>The effect of environmental factors on quality and quantity of the yield in protected and open fields.

#### Tomato Program

The tomato breeding program is directed towards creation of cultivars for early production and mid-season production for fresh consumption and for processing. As a result of long-lasting breeding program the team works with valuable

genetic materials derived from interspecific hybridisation between Lycoprsicon esculentum and wild species such as L. pimpinellifolium, L. chilense, L. pennellii, and L. peruvianum. These species possess complex resistance to pathogens such as Cladosporium fulvum, Fusarium oxysporum f.sp. lycopersici, Leveillula taurica, Corynebacterium michiganense, and Verticillium dahliae. Major emphasis is to create gene resources, combining high biological value with disease resistance to new races of economically important pathogens. As a result of crosses between L. esculentum and L. chilense indeterminate tomato lines were created by the joint efforts of tomato breeders and the Vegetable Fruit Quality Lab teams. Lines were divided into three types according to concentration of b-carotene based on total pigmentation in the fruits: 80-90%, 50-60%, and about 30%. The fruits also possess comparatively high Vitamin C content reaching up to 57 mg per 100 g fresh tissue. A number of gene sources with high level of lycopene (over 10 mg) were created at the Tissue Culture Laboratory as a result of in vitro callus treatment with sublethal doses of gamma rays  $Co^{60}$ . In the same laboratory effective procedures were developed for micropropagation, calligenesis, organo-genesis, and regeneration of valuable breeding lines, cultivars, and F, tomato hybrids. Tomato hybrid forms and stable lines between L. esculentum and L. peruvianum and L. pennellii were created, as a result of embryo rescue technology. A system was developed for in vitro selection of tomato forms tolerant to the fungal pathogen Alternaria solani using its culture filtrates. In the last two years a successful method was developed for genetic transformation by Agrobacterium tumefaciens, which resulted in the creation of transformed tomato lines with heavy metal tolerance. At the Virus Immunity Laboratory, the most promising lines with high biological value are transformed with tomato mosaic virus (TOMV) and cucumber mosaic virus (CMV) resistance. A large part of the tomato breeding materials for fresh consumption and processing are assessed for their chemicotechnological and sensory properties at the Vegetable Fruit Quality and Processing Laboratories. Genetic diversity of the exploited materials of tomato is characterized and identified at the Molecular Genetics Lab using molecular markers.

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The aims of a joint program with the Cytogenetics Laboratory are to incorporate a malesterile gene in valuable breeding lines and cultivars as well as to investigate the expression of dominance and recessiveness responsible for fertility and. sterility in hybrids and backcross progenies. Incomplete dominance and lethality were expressed in some of the homozygous genotypes. To prevent flower abortion in winter and early spring, the Physiology Laboratory licensed some substances, which influence earliness and yield.

A comprehensive system of soil and substrate analysis was elaborated at the Plant Nutrition Laboratory in order to diagnose soil and substrates nutrient status. Monitoring of nutrient needs of tomato grown as soil and soilless culture is being carried out in order to recommend the optimal norms for fertilization. Relationships are established between the nutrient levels and the quality of production. In collaboration with Plant Protection Unit the impact of vesicular mycorrhizae (VAM) fungi is being investigated on the growth, development, and productivity of tomato as well as the use of biological agents against soil-borne pathogens and root-knot nematodes as an alternative to methylbromide as a treatment for soil disinfection in greenhouses.

Biomethods are being improved by searching for new biological agents. Several entompathogenic fungi (Aschersonia sp., Verticillium lecanii), parasites (Encarsia formosa, Aphidius matricariae, A. ervi) and predators (Envtoreiulus persimilis, Macrolophus costalis, M. nubilis, and Amblyseius sp., Orius niger) are used as bioregulators to ameliorate the density of economically important pests on tomato: Trialeurodes vaporariorum, Myzus persicae, Aphis nasturtii, A. gossypii, Thrips tabaci, and Frankliniella occidentalis. The antagonistic activity of Trichoderma sp., Gliocladium and Ånterobacter cloaceae is investigated. Procedures are elaborated for production of soil-borne and aerogenic fungal pathogens.

#### Pepper Program

In the course of over 70 years great diversity of genetic materials has been created and maintained, mainly based on unique local forms typical for the region of Bulgaria. They were improved by methods of conventional breeding. Some of these local cultivars, due to their valuable characteristics, are still in production and are also initial materials for creation of new cultivars, responding to contemporary requirements. At present the pepper breeding team is involved in a valuable collection of over 500 local and introduced accessions.

Contemporary breeding program is focused on the creation of green and red fruited types as well as pepper for grinding (paprika). The breeding program for "green pepper" and "red pepper" emphasizes the creation of lines and cultivars with high biological value (mainly with ascorbic acid content over 120 mg per 100 g for green and over 200 mg for red one) as well as good sensory properties. The desired characteristics for fresh consumption are crispness, succulence, freshness and non-pungency; for processing (for baking, canning, and for juices) they are easy peeling, fleshy pericarp and intensive red color. Attention is also paid to variable nuances of green (light yellow to dark green) and red (light orange to dark red) colors and to various shapes: oblate, edged, bell, blocky, conical tapered, kapia, and slender type.

The objectives of the paprika-breeding program are earliness and uniformity in ripeness as well as enhancement of quality and quantity of total pigments and their long storage. Red pepper lines with over 300 ASTA units are now established. One of the breeding directions is to search for or create forms with stable pigment content after frosting.

At the Plant Tissue Culture Laboratory procedures are optimised for obtaining haploids in anther culture, derived from local cultivars of *Capsicum annuum*. These procedures will be used for species conservation, maintenance, and creation of homozygous lines. Also effective procedures for micropropagation, callusogenesis, organogenesis and regeneration of valuable forms, lines, cultivars and F, hybrids are developed.

The investigations at the Virus Immunity Laboratory are focused on strain variability of the most widespread viruses on pepper in Bulgaria: tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV). Recently pepper mild mottle virus (PMVV) and eggplant mottle dwarf virus (EMDV) were discovered in greenhouse pepper during a collaborative program with the Plant Virology Institute in Torino, Italy. One of the aims of the team is to introduce TMV resistance in several traditional pepper cultivars. Breeding lines and hybrids resistant to Verticillium dahliae and Phytophthora capsici have been created thanks to a joint program with the Plant Protection Unit. An important aim of the breeding team is to create male sterile analogues of the most widespread commercial pepper cultivars in order to be used in a hybrid program. The requirements of new pepper cultivars to environmental factors are investigated with the Agrotecnology Unit in order to assess their suitability for different growing regions.

#### Cucumber Program

The cucumber-breeding program deals with all types of cucumber species such as long European type, American slicers, mini and pickling cucumbers. A great number of  $F_1$  hybrids of top performance have been created, most of them with predominantly gynoecicus flowering habit, parthenocarpic fruits with dark green to green color, smooth to slightly rubbed surface, tolerant to *Sphaerotheca fuliginea*. Several slicer  $F_1$  hybrids types used as the seed parent combine CMV tolerance and predominantly gynoecicus flowering habit combined with excellent taste and flavor of widespread monoecicus cultivars used as a pollen parent.

As a result of collaboration between the Virus Immunity Laboratory and Plant Virology Torino a cucumber virus was identified as a new putative tombusvirus species provisionally named cucumber Bulgarian latent virus (CBLV). Also, EMDV on cucumbers is announced for the first time in Bulgaria.

For creation of breeding materials with complete homozygosis in respect to some valuable characteristics, haploid cucumber plants were produced with the joint efforts of the Department of Plant Genetics, Breeding and Biotechnology at Warsaw Agricultural University, Poland and the Cytogenetics Laboratory. Embryos were induced by gynogenesis and cultured in vitro. The subsequent cytological analysis determined them as monohaploids. Several doubled haploids and genome chimeras were obtained by colchicine treatment. Interspecific hybrids were created in vivo between C. sativus and C. melovar. agrestis subsp. sikimensis to increase genetic diversity. The Physiology Laboratory licenses several complex substances which induce male flowers in gynoecious lines necessary for their reproduction as parents in hybrid programs.

The intensive breeding program matches up with continuous new demands in term of disease resistances. A new trend of the current plan is to combine the CMV resistance with resistance to *Sphaerotheca fuliginea* and *Pseudoperonospora cubensis*. The team disposes with lines, possessing complex resistance to these three pathogens.

#### Cabbage Program

Bulgaria appears to be a second center of origin for *Brassica oleraceae* var. *capitata* (heading cabbage). Cultivars developed at the Institute possess unique taste characteristics, appropriate for fresh consumption and processing. Two cultivars, 'Kiose' and 'Balkan', derived from a local accession, were widely used for years for fresh use and for kraut. 'Besapara' is the only cultivar in Bulgaria for early spring production by autum planting.

The objectives of the breeding program are to release early white cabbage cultivars for spring sowing and broccoli with high ascorbic acid content and resistant to hollow stem. Collaboration programs with the Plant Tissue Culture and Cytogenetics Laboratories strive to increase the genetic diversity in heading cabbage using in vivo and in vitro muthagenesis mainly to separate male sterile forms. Also procedures are optimised for obtaining haploids in anther culture.

As a result of several years screening of valuable gene sources from Brassica oleracea var. capitata, var. sabauda and var. italica, the breeding team has created several breeding lines resistant to Peronospora parasitica. Current investigations are dealing with complex resistance towards three pathogens: P. parasitica, Alternaria brassicicola, and Xanthomonas campestris pv. campestris. The conducted tests with entomologists from the Plant Protection Unit revealed resistance to Breyicorine brassicae in white cabbage. Search for resistance to Mamestra brassicae, Pieris brassicae and Pieris rapae is an aim of the team. A project with the Agrotechnology Unit deals with introduction of organic farming systems appropriate for Bulgarian cultivars of white cabbages.

#### Bean Program

Phaseolus bean cultivars of the Maritsa Institute carry the genetic background of valuable local forms and accessions. They are orientated mainly in two directions: fresh consumption and processing. Most of the cultivars are bush type, with oval or cylindrical, stringless, straight and smoth skinned pods, with dark green, light medium green or yellow color, resistant to Pseudomonas phaseolicola and bean common mosaic virus (BCMV). Recently some cultivars were released for home gardeners with runner growth habit, flat pods and superb bean flacer.

Serious obstacles for bean production are virus diseases. A breeding program for virus resistance is carried out together with the Virus Immunity Laboratory to investigate virus variability and create resistant gene resources. The team develops breeding lines and cultivars resistant to ONV, temperature dependent necrotic strains of bean common mosaic virus (BCMV) as well as to bean common mosaic necrosis virus (BCMNV). The collaboration program with the Vegetable Fruit Quality Laboratory is to release breeding lines combining viral and bacterial resistance with good sensory characteristics.

The Cytogenetics and Physiology Laboratories developed a procedure based on gamete selection for obtaining high temperature-tolerant genotypes in bean. Polyploids were induced in *P. vulgaris*, *P. coccineus*, and *P. acutifolius* to obtain fertile amphidiploids from interspecific hybrids in *Phaseolus*.

The collaboration program between the bean breeders and entomologists from the Plant Pathology Units aims to investigate the level of susceptibility toward *Acanthoscelides obtectus* in different accessions and cultivars of *P. vulgaris*. A number of lines have been identified that possess pest tolerance. The effectiveness of various phytopesticides applied against bean weevil in open field is under investigation.

#### Pea Program

Pea cultivars of *Pisum sativum* created at Maritsa Institute are widespread in Bulgaria. They satisfied the needs for early, mid-early and late production of peas mainly for canning and freezing. Most of the cultivars are wrinkle-seeded. The new tendencies in the breeding program concern the development of super early cultivars, uniform ripeness, multipoded, with increased number of peas; resistance to the most spread viruses and tolerance to high temperature and drought. Models for sensory evaluation of various breeding materials, adapted to various uses, are carried out at the Vegetable Fruit Quality Laboratory.

#### Onion Program

Since Bulgaria is considered to be a secondary center of origin for onion, most of the cultivars created in the Institute possess valuable characteristics of local accessions and forms. They are distinguished by good keeping quality due to firm, tightly adhering skin and thin neck; by attractive yellowbrown to light brown color; by variability in shape from globular to slightly flattened globe; for growing by direct seeding or by sets.

The Institute has significant achievements in hybrid breeding and hybrid seed production in onion. The present breeding program is focused on creating male-sterile lines and their maintainers, with strong pungent to sweet taste. Attention is paid to lines with white colored bulbs and high dry matter content suitable for dried onion production as well as to gene resources, resistant to *Botrytis* sp.

#### Potato Program

Bulgaria is at the border area of optimal conditions for potato growing. The most important limited factor for potato production is aphids as virus vectors causing degeneration of seed potatoes. In this respect the Institute is a pioneer in elaborating a strategic breeding program in the 1960s for the creation of virus-resistant cultivars and technology for virus-free seed production.

Genes for virus resistance were transferred from different wild species such as Solanum acaule, S. demissum, S. sisimbrifolium, S. chacoense, S. stoloniferum to S. tuberosum materials. Valuable breeding lines and cultivars ('Iverze' and 'Nadejda 25') were created with high productivity, earliness and good organoleptic properties. In order to increase the genetic diversity and to create initial materials with complex immunity to virus diseases and late blight (Phytophthora infestans) an International Station was founded in the Rhodope Mountains with Bulgarian and German collaboration. A network for virus-free seed production was elaborated and consisted of tissue culture laboratory for virus-free initial materials, insect-proof greenhouses and highland farms with low aphid infestation.

The current potato program place special emphasis upon introducing cyst resistance to nematodes (Globodera rostochiensis) in valuable and widely used cultivars. The Plant Tissue Culture Laboratory uses experimental mutagenesis to increase genetic diversity, mainly to separate early forms. Serious damages to potato production due to climate changes impose a new trend in breeding program: creation of breeding lines and cultivars tolerant to high temperature and drought. This program is a collaboration of the Physiology and Cytogenetics Laboratories. The potato team is currently working on the creation of specialized cultivars for boiled potatoes and puree, French fries and chips in order to respond to the new requirements of the processing industry in Bulgaria. To ameliorate and to increase the quality and quantity of yield from early potatoes, the Agrotechnology Unit is investigating the application of nonwoven plant covers.

#### PROSPECTS FOR FUTURE RESEARCH

Because of the forthcoming membership of Bulgaria in the European Union there are several important research objectives for future research.

>To transfer the recent achievements of European knowledge in the field of plant genetics, fruit quality, integrated pest management (IPM) to Bulgaria. This will be achieved by courses, conducted by prominent European lecturers and by shortterm visits of young PhD students and researchers of the Institute to European Institutes;

> To develop Maritsa Institute as a promoter of Vegetable Crops Improvement Linkage (VCI Inner Linkage and VCI Balkan Linkage) in order to integrate research and researchers in Bulgaria and in the Balkan region;

>To create motivated young researchers, disseminate scientific information and results, and facilitate communication and collaboration between the Balkan countries with similar scientific interests in the field of vegetable science.

One of the contributions of the VCI Inner Linkage will be elaboration of strategy for Bulgarian vegetable science that will be relevant to Europe. The Inner Linkage will be also exploited as a social instrument for elaboration and diffusion of technology and knowledge transfer program for farmers, seed producers and processors. The most important achievement of VCI Balkan Linkage will be to bring in contact scientists from the Balkan region in order to join their research efforts for creation of high quality vegetable technology, based on the existing gemplasm. Wide range of local germplasm possessed by the Institutes in this region will be exchanged, offering newqualities and traits for investigations.



Maritsa Main Entrance House, built in 1960. It is surrounded by rare and valuable dendro species and comprises mainly administrative and library premises, some labs, and offices of researchers. The other Units are scattered in an Arboretrum, in smaller buildings.



Green pepper variety for fresh consumption.



Promising tomato breeding line for fresh consumption.

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Pepper seed production.



Onion seed production.

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## MOLECULAR MEDICINE CENTER - NOVEL CENTER FOR BIOMEDICAL RESEARCH IN BULGARIA

Albena Jordanova, PhD and Assoc. Prof. Ivo Kremensky, MD, PhD

2, Zdrave Str. 1431 Sofia, BULGARIA, Tel: + 359 2 9172 268, Fax: + 359 2 9172469, E-mail: kremensk@medfac.acad.bg, http://www.lmpbg.org

Molecular Medicine Center is a collaborative, multidisciplinary research center at Sofia Medical University established in 2005 with the support of the Specific Support Action Program of the European Union. The Center upgrades and promotes the activities of the Laboratory of Molecular Pathology, the existing national facility for diagnosis and research in the field of human genetics, whose 27-years tradition, expertise, and international links provide solid foundations for future development.

The major goal of the Molecular Medicine Center is enhancing the capacity for biomedical research in Bulgaria. This will be achieved through promoting and developing the interaction between the Medical University's departments and Bulgaria's major diagnostic and research facility in the field of human genetics, the Laboratory of Molecular Pathology (IMP) and stimulation of genetic research in all fields of medicine, focusing on common genetic diseases of particular relevance to the country morbidity profile.

#### BACKGROUND

The Molecular Medicine Centre is built upon: (i) the role of IMP as a national genetics center, its existing capacity and know-how, established links with clinical medicine, and international reputation; (ii) the research expertise of its members and the role of Sofia Medical University as the country's leading tertiary medical institution; (iii) the growing interest of Bulgarian clinical specialists from different areas of medicine in the molecular factors conferring common disorders.

#### The Laboratory of Molecular Pathology

IMP was founded in 1977 as a joint initiative of the Faculty Departments of Pediatrics and Obstetrics and Gynecology, and the Ministry of Health. The founding senior scientists were Luba Kalaydjieva, currently Professor of Molecular Genetics at the University of Western Australia and Visiting Professor at the Faculty of Medicine of Sofia Medical University, and Ivo Kremensky, currently Associate Professor at the Faculty and Head of the Laboratay.

IMP was conceived as Bulgaria's major diagnostic, research and training center for biochemical and molecular genetics. The World Health Organization (WHO) Fund and the United Nation Populations Fund (UNFPA) supported its establishment. Its mission and future development have been carefully charted in consultation with WHO experts, and in compliance with international standards applied to the country's population size, geography, health care system and infrastructure.

The Laboratory is functionally divided into six sections: Molecular Pathology, Biochemistry, Mass neonatal screening (for PKU) and prenatal screening (for Down Syndrome); Prenatal Diagnosis of Inherited Disorders, Cytogenetic diagnosis and Human Reproduction (with Sperm Bank), equipped for biochemical and molecular genetics analyses. LMP as the national centre for diagnosis and prevention of genetic diseases:

LMP conducts two national mass screening programs - of newborns for PKU and galactosemia (since 1977 more that 1 800 000 newborns have been screened), and prenatal for Down syndrome and neural tube defects (since 2000). It offers a battery of metabolic and enzymological assays for diagnosis of over 70 inborn errors of metabolism. It was among the first in Eastern Europe to introduce molecular genetic testing. The Laboratory performs molecular analyses for common aneuploidies, over 30 Mendelian disorders and other DNA-based analyses, including forensic paternity testing. IMP has well-established procedures for transport, reception, processing, and archiving of biological samples. It is managing several national sample collections, ranging in size from hundreds to many thousands, including Guthrie cards from newborns and a DNA bank of families with genetic disorders. The computerized register contains information on over

20,000 families, including >2,000 with monogenic discuss.

The Laboratory initiated and has been actively involved in designing and implementing of the current National Program for Diagnosis and Prevention of Inherited Disorders and Congenital Anomalies in Bulgaria, funded by the Ministry of Health, and in the legislation and policy-making process in the field of healthcare, health insurance and other issues related to genetic testing. During the 27 years of its existence as a national center LMP has gained a comprehensive overview of hereditary pathology, and knowledge of the health care infrastructure of the country. Its close links to clinical medicine have shaped its research profile and trained its staff in communicating with the medical community. In its turn, LMP is known to the medical community countrywide as a reliable, long-standing partner in patient care, and thus the first choice for scientific partnership.

The Laboratory staff members have helped in setting up parents' and patients' associations for Neuromuscular Disorders, Phenylketonuria, Gaucher's Disease, Cystic Fibrosis, Neurofibromatosis and are involved in the activities of these associations, reporting on the advances of genetic research among patients. Major achievements of IMP in studying genetic disorders and population structure of the Roma/Gypsies have been possible due to the fruitful collaboration with the non-profit organisations like the Roma Foundation and the "Health Problems of Ethnic Minorities" Foundation- our partners in ongoing projects related to the Roma population.

## MMC HAS INTERNATIONALLY RECOGNIZED SCIENTIFIC PROFILE AND CONTRIBUTION TO EUROPEAN SCIENCE

Major objectives of the newly established Molecular Medicine Centre are oriented towards research directly related to human health and wellbeing. Its staff members are known to the European scientific community and respected for their contributions to several areas of genetics.

#### The mutational basis of Mendelian disorders.

MMC researchers have a solid reputation in the Medelian disorders research. They were one of the founding members of the World Cystic Fibrosis Genetic Analysis Consortium and currently participate in the European Cystic Fibrosis Consortium and the Charcot-Marie-Tooth Disease Consortium. Their studies of the common single-gene disorders have led to identification of a large number of novel disease mutations. This research has contributed to understanding the genetic landscape of Europe, as well as to the study of genotype-phenotype correlations. They have also made methodological contributions to the field by developing highly sensitive mutation screening (SSCA) protocols, now widely used by many international laboratories.

## Gene discovery:

The longstanding strong emphasis on neurogenetics, in close collaboration with the Department of Neurology, has led to the identification and genetic characterization of four novel disorders of the peripheral nervous system. This work is now expanding into epilepsy and other neurodegenerative disorders.

#### Transition to complex disorders.

The Laboratory of Molecular Pathology has traditional links with the Departments of Pediatrics and Obstetrics due to its focus on Mendelian disorders. This is now changing, with an exponentially growing interest of a wide range of clinical specialists in the genetic basis of common genetically complex disorders. The MMC is involved in research into bipolar affective disorder in collaboration with the Department of Psychiatry. Research into complex disorders is expanding into cardiovascular disorders, which are a major health problem and the leading cause of death in Bulgaria, neurodegenerative disorders, drug abuse, familial cancer, osteoporosis, diabetes and genetic predisposition to complications of pregnancy and delivery.

#### Population genetics:

The key geographic location of Bulgaria makes it an important contributor to understanding the population history of Europe. Publications of MMC staff members in population genetics are widely cited in studies of European populations genetics. We are regular contributors to the European forensic database.

## MMC makes a unique contribution to genetic research and health care in Europe.

The Laboratory of Molecular Pathology under the supervision of Prof. Luba Kalaydjieva has initiated pioneering genetic studies into Europe's most interesting founder population - the Roma (Gypsies), that are currently continued by the MMC. These studies have led to appreciation of the value of this trans-European isolate for genetic research, and are helping health care in EU countries by providing information on novel diseases and founder mutations in newly arriving patients and entire communities.

### Publication record, research funding and international collaborations:

The publication record of MMC scientists exceeds more than 110 highly cited papers in international peer reviewed journals, including Nature Genetics, the American Journal of Human Genetics, and Human Molecular Genetics. The MMC research is supported by grants provided on a competitive basis by national and international research-funding agencies. Currently MMC is awarded with more than 15 national grants (from Medical University of Sofia and the National Science Fund) and participates in 5 internationally-funded projects, including the Muscular Dystrophy Association of the USA; the Neuropathy Association of the USA; National Institute on Drug Abuse, USA; National Health and Medical Research Council, Western Australia and INIAS.

MMC collaborates with multiple European institutions, many of which have been or are currently involved in training MMC staff. The list includes, among others, the Department of Clinical Genetics, Erasmus University, Rotterdam, the Departments of Biology and Neurology, University of Padova, the Department of Human Genetics, University of Wurzburg, the Institutes of Human Genetics at the Universities of Munster and Bonn, the Department of Molecular Genetics, Flanders Interuniversity Institute of Biotechnology (VIB-08), the Center for Human Genetics, KU Leuven, the Department of Molecular Biology and Genetics, Universitá "La Sapienza", Rome, the Life and Brain Center in Bonn, Center of Molecular Medicine, University of Edinburgh, the Department of Psychiatry, University of Diblin, etc.

## MMC PROVIDES UNDERGRADUATE AND POST-GRADUATE TEACHING AND TRAINING IN MO-LECULAR GENETICS

MMC has inherited the LMP traditions in organizing and conducting Molecular Medicine

courses under various European programs (TEM-PUS; Socrates; Leonardo da Vinci). The Center is involved in undergraduate, postgraduate and specialized medical education with four full-time courses in Molecular Medicine and lectures and tutorials in molecular pathology included in the courses of pediatrics, obstetrics, internal medicine and clinical laboratory. MMC participation in undergraduate training, as well as hosting and joint supervision of young researchers from different areas of clinical medicine, will influence the thinking and philosophy of the new generations of medical practitioners.

The Center is also providing specialized courses in Molecular Pathology and in Population Genetics and Anthropology for the Bachelor and Master of Sciences programs of the Biological Faculty of Sofia University, and is involved in teaching at the Medical University of Pleven. MC offers exchange training for students - from other Bulgarian and international universities (from Macedonia and India). It is a major center for postgraduate training of science (MSc and PhD) students with more that 40 graduates in the last five years.

#### MMS INTER-DISCIPLINARY NATURE

MMC is a scientific network of investigators from different departments and research groups in Sofia Medical University. With their expertise they contribute to different fields of medicine, provide access to large numbers of well-characterized patients, and enable joint initiatives in the field of under- and postgraduate medical education.

To broaden the scope of research projects in molecular medicine and include other fundamental studies, MMC collaborates with other institutes in Bulgaria, working in the field of Cell and Molecular Biology, like the institutes of the Bulgarian Acadeny of Sciences, the Institutes of Molecular Biology, Virology, Immunology and Developmental Biology, Cenetics; the Biological Faculty of Sofia University; the Institute of Infectious and Contagious Diseases and others. The joint research efforts lead to improved understanding of the role of genetic research in clinical medicine and provide a critical mass of expertise in various fields of medicine and genetic research. Their commitment promotes novel and dynamic research and creates an improved interactive research environment that attracts and retains young scientists and facilitates dissemination of knowledge.

#### MMC RESEARCH STRUCTURE

Research groups are the main structural unit in MMC. They are formed, based on the specific demands from different Departments of Sofia Medical University and the current trends in genetic research. These are multidisciplinary teams of researchers from different departments of Sofia Medical University and the IMP, working on specific collaborative projects with access to common technical and managerial resources. The current research topics in MMC are:

A Inherited Peripheral Neuropaties (IPN) molecular defects and genotype-phenotype correlations, mapping of novel loci and identification of genes associated with IPN, clinical and genetic characterization of Dominant Intermediate Charcot-Marie-Tooth (DI CMT) disease, and functional genomics of DI CMT type C.

**B** Muscular dystrophies - molecular defects and genotype-phenotype correlations.

**C** Affective psychiatric disorders - large-scale association studies, mapping of novel loci for bipolar/unipolar affective disorders, case/control and trics studies.

**D.** Familial Cancer - searching for genetic predispositions related to familial colorectal and endometrial cancer.

**E** Epilepsy syndromes - molecular defects and genotype-phenotype correlations, mapping of novel loci associated with GEFS+ syndromes.

**F** Genetic predispositions related to drug addiction, autism, reproductive failures, dementias and stroke.

**G** Ophthalmologic disorders - molecular defects and genotype-phenotype correlations, mapping of novel loci and identification of genes associated with Retinitis pigmentosa and glaucoma.

H Development of new DNA based methods for identification of an uploidies.

I Population genetics - molecular characterization of the main ethnic groups of the Bulgarian population, etc.

The Molecular Medicine Center is coordinated by Assoc. Prof. Ivo Kremensky and two local goveming bodies: the Scientific Board and the Project Management Committee. The Scientific Board determines the directions and guidelines of MMC research. The Project Management Committee ensures the quality and timely implementation of the deliverables and milestones outlined in the individual research projects carried out in MMC. The Center is overseen by an Advisory Board, composed of senior scientists affiliated with national and international institutions outside the Medical Faculty.

The research performed in MMC is supported by three main core facilities: Biobanking, Genomics and Bioinformatics. The Biobanking core has the capacity to provide a number of specialized high quality services, such as genomic peripheral blood lymphocyte, plasma and DNA isolation from large numbers of samples, and cryopreservation for longterm secure data storage and retrieval. The Genomics core offers an up-to-date technology platform for cost effective and mid- to high throughput DNA/RNA analysis. The Bioinformatics core ensures access to various databases and analysis software and organises the data produced by different individual projects into well-annotated databases. This core makes the data available to the Bulgarian scientific comunity through the development of user interfaces/application programming interfaces in combination with powerful analysis tools. The bioinformation actively trains researchers and students in biomedical sciences in this novel biomedical discipline, so they can use these data efficiently.

## MMC PERFORMS ACTIVE NETWORKING AND DISSEMINATION OF KNOWLEDGE

MMC has established a local network within Sofia Medical University by maintaining monthly joint seminars and joint supervision of PhD students and postdocs affiliated to the different departments, who perform the molecular genetic part of their research in the MMC.

MMC is subscribed to the most important national and international journals in Biomedical science and serves as a platform for dissemination of research achievements and project results among a broad range of specialists. Annual open-door days for visits in the research facilities are organized to attract the interest of young people (students and pupils from high schools) and all interested in molecular medicine. Special Seminars are held for media representatives on hot-topic issues in genetic research, like cloning, stem-cells research and opportunities, utilization of GVD - advantages and disadvantages. The purpose is to increase public awareness about genomic and postgenomic research and its application for better quality of life.

Major responsibility of the professionals in MMC is **dissemination of the legislation documents** of the European Authorities and policy recommendations. **Expert help** to governmental institutions and policy makers for creation of laws and regulations on genetics-related issues is a part of the MMC program. The MMC experts advise Bulgarian national policy makers in the prevention of genetic diseases and implementation of the European standards in the research related to human health and quality of life. Special emphasis is laid upon contacts with policy makers to adapt Bulgarian laws regarding genetic testing and human health in accordance with European Law. A website is in process of establishment at the MMC to allow access to information - both general and projectspecific.

The Molecular Medicine Center contributes to Bulgaria's research and technological capacity in the field of Life sciences, Genomics and Biotechnology, allows development of self-sustaining research programs and adds value to the European Research Area.

## GENETIC SERVICE FOR CYSTIC FIBROSIS IN BULGARIA

### Alexey Savov, PhD; Albena Jordanova, PhD and Assoc. Prof. Ivo Kremensky, MD, PhD Laboratory of Molecular Pathology, Sofia, Bulgaria

Laboratory of Molecular Pathology is the National Centre providing molecular diagnostics, prevention and genetic counseling of Cystic Fibrosis (CF) in Bulgaria. Since 1985 more than 350 CF families have been analyzed. The molecular screening approach includes a battery of highly informative and sensitive methods for DNA analysis, such as Heteroduplex analysis, Single Strand Conformation Polymorphism Analysis (SSCA), direct DNA sequencing and haplotype analysis.

Our mutation detection strategy has allowed detection of 92% of molecular defects in Bulgarian CF patients. So far, we have identified 37 different mutations of which 13 were novel mutations (Savov et al., 1994a, b; 1996). We were the first to demonstrate that double mutant alleles are not a rare finding in CF with serious implications for molecular diagnosis and genetic counselling (Savov et al., 1999).

Our genotype-phenotype correlation studies revealed that Meconium ileus is a clinical complication significantly associated with 1677delTA mutation and that R347P mutation causes CF characterized by pancreatic insufficiency and pulmonary disease. We performed population genetics study of CF in Bulgaria via comprehensive mutation analysis and construction of intragenic microsatellites of 262 CF alleles. Deletion delF508 accounted for 100% of Gypsy OF alleles, which thus differed significantly from both Bulgarians and ethnic Turks. In Bulgarian and Turkish CF patients, 92% of the mutant alleles were identified, yielding a total of 25 different mutations, of which only 7 occurred at frequencies higher than 1%. Genetic distances and population trees demonstrated that in the southeastern tip of Europe, the overall distribution of CF mutations and polymorphic haplotypes is very close to that of Mediterranean populations, with a high frequency of N1303K and G542X, a large number of rare mutations and a prevalence of the 23 31 13 haplotype in association with deltaF508 (Angelicheva et al., 1997).

Apart from the CF research, we provide preand postnatal diagnosis of the disease. So far, we have performed prenatal analyses in 139 CF families and diagnosed 35 affected, 62 carriers and 42 healthy fetuses.

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## NEUROPSYCHIATRIC GENETICS RESEARCH GROUP

## Radka Kaneva, PhD; Veselin Chorbov, Mina Angelova, MSc; Momchil Nikolov, MSc - Laboratory of Molecular Pathology, Tel: + 359-2-9172473, Fax: +359-2-9172469; E-mail: kaneva@medfac.acad.bg Vihra Milanova, MD, PhD; Vessela Stoyanova, MD, PhD; Rositza Vladimirova, MD; Spiridon Alexiev, MD; Nikolina Djurova, MD, MSc - Department of Psychiatry, Sofia Medical University

Affective disorders are severe and common psychiatric diseases with two main clinical forms: Bipolar and Unipolar Affective Disorders (BPAD and UPAD). BPAD is characterized by major depressive episodes alternating with phases of mania (bipolar I disorder) or hypomania (bipolar II disorder). UPAD is characterized by recurrent major depressive episodes alone. Affective disorders are highly prevalent and associated with severe social disability and considerable mortality both with major economic impact.

The etiology of mood disorders is multifactorial involving genetic as well as environmental factors. Traditional methods such as family, twin and adoption studies first have provided cumulative evidence implicating genetic factors in the vulnerability for affective disorders. Using these methods however, it is difficult to delineate which genetic variables are involved as well as the exact mode of transmission of the disease. Linkage and Association methodologies are currently applied in order to identify potential susceptibility genes.

The group of neuropsychiatric genetics works in the field of genetics and aetiology of affective disorders and schizophrenia in the last 12 years. It consists of psychiatrists from First Psychiatric Clinic, Alexander University Hospital and Department of Psychiatry, Medical University -Sofia and molecular biologists from the Laboratory of Molecular Pathology. The founding of the team was encouraged by Prof. A. Jablensky and Prof. L. Kalaydjieva to study the genetic factors contributing to the aetiology of major psychiatric disorders in the Bulgarian population. The research group participated in many national and international research projects. The clinical team lead by Assoc. Prof. Dr. Milanova works in the field of biological psychiatry and clinical psychopharmacology - treatment of bipolar affective disorder, depression, and personality disorders. The psychiatrists have been trained in using various research diagnostic interviews.

The Fogarty Research Foundation supported in 1992-1994 a research project on Foetal Neural Development and the aetiology of Schizophrenia. The work was further extended by a collaborative project with University of Western Australia, funded by the Stanley Foundation 1995-1996 research award: Morphometric and Neural Connectivity Characteristics of Fetal Brains at Genetic risk for Bipolar Disorder.

The collection of families with multiple affected members, suitable for linkage analysis was initiated in 1992 and supported by a project, funded by the Ministry of Education and Science of Bulgaria. In 1994 the research team was invited to join the ongoing European initiative under the BIOMED1 program of the EU: European Collaborative Study of Affective Disorders: Interaction between Genetic and Psychosocial Vulnerability Factors (1991-1994). The coordinator of the project Prof. J. Mendlewicz, Erasmus Hospital, Free University of Brussels, Belgium, formed a research consortium of 13 partners from 8 European countries. The Bulgarian contribution was extended with our participation in the second European project under BIOMED2 program: European Collaborative Study on Molecular Genetics in Affective Disorders (Genes in Affective Disorder).

The patients included in the project were recruited from two Bulgarian Social Services for Mental Health in Sofia and the Psychiatric Clinic of Alexander University Hospital, Sofia. The Bulgarian association sample consists of 150 patients with affective disorder (Bipolar and unipolar) and 150 controls matched accordingly by sex, age and ethnicity.

Standardized validated diagnostic interviews (SCAN, DIP) criteria are performed by trained psychiatrists for all patients giving diagnosis based on DSM-IV. The operational checklist for mental disorders (OPCRIT) and Family Interview for Genetic Study (FIGS) are used in addition. The phenotype data is stored in computerized clinical databases.

The BIOMED projects focused on psychosocial parameters relevant to the phenotype of affective disorders evaluated with reliable instruments: Social adjustment and self-esteem self reported scale and Temperament and Character Inventory interview of (Cloninger, 1992). Psychosocial evaluation is done in addition in patients in remission and healthy controls. The interviews were translated from English to Bulgarian and tested among a pilot group of healthy controls. The interview has been given to all newly recruited patients and the parents and sibs of affected probands from the trios.

After informed consent, venous blood is collected from all patients, family members and healthy controls. The DNA bank at present contains more than 500 samples of large families with affective disorder, small probands/parents trics and case/ controls collection.

Under the BIOMED and BIOMED2 projects the Bulgarian case/control sample was included in a large European database, with more than 600 patients with Bipolar Affective disorder, 600 patients with Unipolar Affective disorder (recurrent depression) and 1200 healthy controls.

Candidate genes were selected, coding for receptors of the major neurotransmitters (GABRA1, GABRA3, GABRA5, DRD2, HT2C, HT2A); their transporters (dopamine and serotonin transporters) or key enzymes such as MAOA, NOS1, PLA2A, COMT. Genotyping of selected polymorphisms was completed and association analysis was performed for all selected markers in candidate genes in our sample. The results from the European studies were published in more than 15 articles in international journals.

After the completion of the two European collaborative projects the research team carried on with strengthening and expanding the clinical team, recruiting more families with affective disorder. A unique collection of families, clinically well studied and suitable for genetic linkage analysis was gathered, including 7 small families of Bulgarian origin and 3 extended multigenerational Roma families.

In case of complex disorders, a complete genome scan is needed to estimate the number of contributing genes, as well as the magnitude of the gene effect for which we have no prior knowledge. A complete systematic genome scan in these families was performed in the Max Delbrück Center for Molecular Medicine in Berlin. Statistical analysis of the results from the genome scan was performed in collaboration with the group of Prof. Wienker at the Institute of Medical Biometry, Informatics and Epidemiology (IMBIE), University of Bonn.

The findings of the large linkage study of bipolar affective disorder (BPAD) that involved genome-wide analysis of 52 families of Spanish, Roma and Bulgarian descent, and further fine mapping of the 1p34-p36, 4q28-q31, and 6q15-q24 regions confirm regions 4q31 and 6q24 as BPAD susceptibility loci and 1p35-p36 is proposed as a new putative

locus requiring confirmation in replication studies. The results should facilitate the search of potential susceptibility genes for BPAD in the candidate regions, performing systematically linkage disequilibrium (LD) mapping.

To continue the work on the Gypsies, a genetically isolated founder population, we have been supported by a Research Grant from the NHMRC, Australia (2005-2007). The objectives of the project include the collection of additional large Roma families with BP, as well as 150 probands/parents trios to serve for replication sample. The first step of verification the positive genome scan data using a denser genetic map and linkage analysis in the existing expanded families is underway. The replication in large BP families from other Gypsy subisolates (groups) and identification of a candidate region harbouring a shared founder mutation will be followed by fine mapping and linkage disequilibrium (ID) analysis.

In parallel the Research team received a grant by the National Science Fund at the Ministry of Education and Science of Bulgaria under a new National Research Program "Genomics". The research project is a natural follow up of our work on genetics of affective disorders. In addition to the main stream of work on fine mapping and looking for genes, contributing to the actiology of BPAD, we focus on the elucidation of the relationship between affective disorders and epilepsy. The unique finding of a subset in the Roma families with BPAD with multiple cases of epilepsy in several generations and comorbid idiopathic epilepsy and affective disorder serves as a starting point in the project. Other Roma families with affective disorders and epilepsy have been recruited during fieldwork among Roma groups in Bulgaria.

A multidisciplinary team was formed, including psychiatric, neurological and genetic units, to fulfil the aims of the project. The genetic and psychiatric teams have already been in close collaboration in previous studies on affective disorder. A leading neurologist, well experienced in the genetics of neuronuscular disorder in Gypsies, Assoc. Prof. Ivailo Tournev formed a team of neurologists and neuropsychologists for detailed clinical study of epilepsy. Assoc. Prof. Vihra Milanova leads the psychiatric team and Dr. R. Kaneva is the leader of the genetic team, as well as a coordinator of the project. International collaboration on the project involves leading experts in epidemiology of epilepsy (Prof. J W Sander, Department of Clinical and Experimental Epilepsy, UL Institute of Neurology); psychiatry (Prof. A. Jablensky, Centre for Clinical Research in Neuropsychiatry) and genetics of Roma population (Prof. L. Kalaydjieva, WAIMR and UWA Centre for Medical Research, University of Western Australia).

## Selected publications of the research team 2003-2005

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## MADE IN BULGARIA WITH EUROPEAN SUPPORT

## SELECTIVE SCREENING FOR DIAGNOSTICS OF INHERITED METABOLIC DISEASES IN BULGARIA

Maria Ivanova, MSc; Ivanka Sinigerska, MSc; Irfet Hasanova and Assoc. Prof. Ivo Kremensky, MD, PhD

Laboratory of Molecular Pathology, Medical University, Sofia 1431, BULGARIA

Laboratory of Molecular Pathology offers modern and widely applicable genetic services for diagnosis and prophylactics of inherited metabolic diseases (IMD). The highly effective methods performed in the Laboratory of Molecular Pathology afford opportunities for reliable specialized genetic analyses for other Balkan countries, which need genetic service.

The Laboratory of Molecular Pathology provides services for 1 specialised clinic, 5 medicogenetic counselling and more than 70 paediatric and 119 neonatal units in Bulgaria. The laboratory performs: 1) Mass neonatal screening for PKU (up to now 1 700 000 newborns were screened, 86 patients with classical PKU were detected); 2) Selective biochemical screening for over 70 inborn disorders of metabolism – up to now 611 of 8592 patients were precisely diagnosed; 3) Prenatal metabolic and enzymatic diagnosis (up to now 43 prenatal diagnoses for different metabolic disorders were performed).

#### SELECTIVE SCREENING PROGRAM

In the Laboratory of Molecular Pathology about 400 patients are tested annually by selective screening program.

It is obvious that the mass neonatal screening approach could ensure diagnostics and prophylaxis in a restricted proportion of diseases (in Bulgaria - 2, in Europe - about 20). For all the remaining disorders, an alternative approach of selective screening is applicable. The basic criterion for selection in a Bulgarian selective screening program is the clinical symptoms and signs constellation. To achieve this, the material for analysis must be accompanied by a detailed clinical and laboratory information. In case that a carrier status determination and prenatal diagnosis are desired, it is mandatory that besides the detailed clinical, genealogical and laboratory information, a definitive clinical diagnosis is required together with DNA samples from sufficient number of family members.

#### Analytical Approach and Methods

It is typical for the Bulgarian selective screening program that a comprehensive range of analytical methods and techniques is used.

The analytical programme includes three-stage systematic approach. Semiquantitative methods (Thin-layer liquid chromatography-TLC, two-dimensional thin-layer liquid chromatography - 2DTLC) for urine metabolites assay are in use at the first stage. The second stage includes quantitative (highperformance liquid chromatography - HPLC, gas chromatography - mass spectrometry - GC/MS) methods and the third stage explores methods for enzymatic activities assay in biological fluids and tissues (see Figure 1). Tandem mass spectrometry will be introduced into analytical approach scon.

All the methods and results obtained by them are under strict internal and external laboratory control.

The laboratory participates in European external quality control - ERNDIM Diagnostic Proficiency Testing. During the period of 25 years, 8592 patients at high risk were included in the selective screening program. In total 611 patients (7.1%) were diagnosed in the basic groups of inherited metabolic diseases: Aminoacid disorders - 44; Urea cycle defects-13; Organic acidurias -53; Fatty acid oxidation disorders - 12; Peroxisonal disorders -9; Disorders of the renal transport - 61; Lactic acidurias - 5; Carbohydrate disorders-131; Glycoprotein degradation - 7; Mucopolysaccharidoses - 54; Ganglioside disorders -150. Our results can be assessed as reasonably good and completely comparable to the data of the leading European centres.

Significant progress in the diagnostics of inherited metabolic disorders in Bulgaria has been made since 1990. This is associated with the progressive introduction of DNA analysis. Thanks to DNA analysis it became possible to confirm the diagnoses of MCAD (A985G), galactokinase deficiency (P28T) and phenylketonuria - most common mutations in Bulgarian population.

PC network and specialised software are in use for the selective screening program.



**Figure** 1. Analytical approach to diagnostics of inherited metabolic diseases - selective metabolic screening: All steps are chosen depending on the clinical picture and results from the previous steps of the analysis.

## DNA ANALYSIS FOR SOME OF THE NEUROMUSCULAR DISORDERS IN BULGARIA

Albena Todorova, PhD; Bilyana Georgieva, PhD; Tihomir Todorov - Laboratory of Molecular Pathology, University Hospital of Obstetrics and Gynecology, 2, Zdrave Str., Sofia 1431, Bulgaria

**Application area:** Diagnostics and prophylaxis, prenatal diagnosis and carrier status determination for some of the most severe, incurable neuromuscular disorders were successfully introduced in Bulgaria.

Duchenne/Becker muscular dystrophy (DMD/BMD) [OMIM # 310200; 300376] is the most common inherited neuromuscular disorder, affecting 1:3500 live male births. The DNA diagnostics of this severe, progressive muscular dystrophy with onset early in childhood, invalidization and death around the second decade of life, was introduced in Bulgaria in 1990. It became possible after the enormous efforts of several neurologists in the country, who performed precise clinical research and described in detail the clinical findings in hundreds of patients, affected by neuromuscular disorders. A group of patients with clinical diagnosis susceptible with DMD/BMD was selected and subjected to DNA analysis. Dr. J. Bronzova introduced first trials for genetic tests in Bulgaria thanks to a very fruitful collaboration with the Sylvius laboratory, Leiden University Medical Center.

Afterwards, several novel and modern methods for DNA analysis of DMD/BMD were developed and introduced for routine analysis in our country by Dr. A. Todorova. This became possible after the establishment of a very profound collaboration with the Laboratory of Human Genetics, University of Padua (prof. G.A.Danieli) and with the Neuromuscular Center, University of Padua (Dr. Marina Fanin, prof. C. Angelini). Long-term specializations were performed in these leading laboratories in the field of diagnostics and prophylaxis of neuronuscular disorders.

Recently, the newest method for DNA analysis of the whole dystrophin gene, named multiplex ligation-dependent probe amplifica-tion (MLPA) method, [www.mlpa.com] was applied also to a number of DMD/BMD Bul-garian patients. This method permits deletions detection along the whole gene sequence, as well as detection of duplications. Moreover, it is also possible to analyze females and directly to determine their carrier status.

This modern diagnostic approach was introduced in Bulgaria after the specialization of Dr. A. Todorova, as a fellow of the Alexander von Humboldt Foundation in the Institute of Human Genetics, Münster (Dr. N. Bogdanova, prof. B. Dworniczak and prof. J. Horst). Very useful and profound collaboration was established after this specialization between both institutes. A number of Bulgarian patients and families benefit from this cooperation.

Thanks to the above-mentioned professional trainings and the subsequently established collaborations, nowadays in Bulgaria we can offer to all DMD/BMD families the most recent and modern diagnostic approaches for DNA tests.

Up to now 259 DMD/BMD families were registered in our database. The performed screening for mutations in our patients revealed about 70% deletions of one or more exons. Recently, 6 duplications (dupl. 48-50, dupl. 8-11, dupl. 2-33, dupl. 13-40, dupl. 2-10 and dupl. 8-13) were also found by MLPA (Figure 1). Moreover, 3 point mutations (exon 16 c.1975G>T, p. Glu659X; exon 41 c.6053delC and exon 54 c.7961A>G, p. Asp2654Gly) and a small deletion of 16 bp in exon 44 were also described in our sample.

Subsequently, the genetic analysis of some forms of Limb-girdle muscular dystrophies (LGMDs) became possible in Bulgaria. Several European studies showed that in Gypsy population LGMD type 2C [OMIM # 253700] has been caused by a single mutation in the g-sarcoglycan gene, p.C283Y. An extensive field work in the Gypsy areas was performed in our country by Dr. I. Tournev and his team to search and clinically examine LGMD2C patients. Several affected individuals were found, most of them located in the Northeastern part of Bulgaria. The genetic analysis demonstrated that in all cases the disease was provoked by the same mutation p. C283Y in the q-sarcoqlycan gene.

In addition, several screening programs were performed in the Gypsy community over the whole territory of the country in order to calculate the carrier frequency of this mutation in the Gypsy population. The results are presented in Figure 2. Some regions showed very high carrier frequency like Razgrad 8.70%, Silistra 5% and Dobrich 4.55%.

The blood samples were taken after written informed consent, and genetic coun-seling was offered to each participant before and after the performance of the DNA test.

Another form of LGMD, type 2A [OMIM # 253600] became possible to be analyzed in Bulgaria after the collaboration with the Institute of Human Genetics, University of Würzburg (prof. C.R.Mbller), supported by the Alexander von Humboldt Foundation. This analysis revealed some novel mutations in the calpain 3 gene, causing LGMD2A in Bulgarian patients (Figure 3). Moreover, it was found that more than 40% of our patients are homozygous for the mutation 550delA and 70.6% carry it at least on one of the alleles. Interestingly, we detected also a large deletion of exons 2 to 8 in two unrelated families, most probably caused by independent mutation events.

The collaborations with the groups of prof. Muïler in Würzburg and prof. Horst in Münster, both supported by the Alexander von Humboldt Foundation, gave us also the opportunity to learn and apply in Bulgaria the routine genetic tests for some rare forms of neuromuscular disorders, like LGMD1C [OMIM # 607801], LGMD1B [OMIM # 159001] and EDMD-AD [OMIM # 181350] and myotonic dystrophy (DM1) [OMIM # 160900].

The presented results and achievements had not only a scientific interest, but they were also of great importance for the affected families and their offsprings. More than 60 prenatal diagnoses were performed so far and a number of healthy children were born.

#### Publications related to the present results:

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16. Georgieva B., Todorova A., Tournev I., Plageras P., Mitev V., Kremensky I. (2005) 550delA Mutation in the Calpain 3 (CAPN3) Gene: DMD/BMD, SMA or LGMD2A - Clinically Misdiagnosed Cases. Am J Med Genet 136A: 399-400.

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Figure 1. MLPA analysis of the dystrophin gene. The duplication of exons 48-50 is presented in comparison to the control sample. Arrows point the duplicated exons.



Figure 2. Geographic distribution of the mutation p.C283Y in the Bulgarian Gypsy population. Underlined numbers give the registered LGMD2C Gypsy patients per region. The percents provide the detected p.C283Y carrier frequency in each region. Arrows give the Gypsy influx on the Balkans.



Figure 3. CAPN3 gene mutations detected in the Bulgarian sample of LGMD2A patients. The number of patients carrying the same mutation is given in parenthesis.

## METHODS AND TECHNOLOGIES FOR MANUFACTURE OF ENZYMES -ALPHA-AMYLASE, XYLANASE, CELLULASE

Contact Address: Mr. Vladislav Jivkov

Applied Research and Communications Fund (ARC Fund), 5, Alexander Zhendov Str., 1113 Sofia, Bulgaria Phone:+359 2 986 7557, Fax:+359 2 9801833, Email: vladislav.jivkov@online.bg

#### Application Area: Food - Agro Industry.

The technologies offered by the Bulgarian research institute are for the manufacture of 3 enzymes - alpha-amylase, xylanase and cellulase. Three different strains are developed, but technological equipment is the same. During the technological process these strains are put in different specific conditions defined for each of them. Different source materials are used. The proposed technologies are not of the GMO type. They are already realized in industrial conditions in Bulgarian factories and prove to work. The enzyme production can be set in different levels of purification of the end products. The enzyme products correspond to the European quality and safety standards. The equipment used is standard for production of bioproducts. No technological problems were met during the production tests in Bulgaria.

Innovative aspects: Innovative strains put in specific defined conditions, Content of the medium used in the manufacturing process.

#### Main advantages:

• Cheap source materials proper for the European region are used: Alpha-amylase - partially hydrolysed farina made of corn, potatoes or rice; Xylanase - corn-cobs; Cellulase - wheat straw which leads to reduced price of the end products.

• About 50% of the price of the end product is formed by the source material.

• These technologies use standard and already existing equipment for the manufacture of bioproducts.

No waste is generated.

## GRANULATED APPLE AND CITRUS PECTINS

Laboratory of Biologically Active Substances - Plovdiv 95, "Vasil Aprilov" Blvd., 4002 Plovdiv Phone: +359 32/45 21 40, 32/44 19 29, Fax: 032/44 01 02 e-mail : LBAS@plov.omega.bg

Application area: Food industry, Human health.

The pectic substances are acid polysaccharides. They are well dissolved in water and give viscous solutions. They are widely applied in food industry as hydrocolloids because of their gelling, emulsifying and stabilizing properties. Pectic substances are found in all fruits and vegetables. They are the most important soluble dietary fibres in daily food of a man. Biological activity of pectic substances increases when they are isolated from the plant raw material. For this reason industrial production of pectin from apple and citrus pressings.began long time ago. The pectin actively affects some physiological processes in the human constitution. It participates in metabolism of lipids, bile acids and carbohydrates. It is recommended for prophylaxis and treatment of ulcer and gastritis, cardiovascular disease, binding and excreting of heavy metals and radioactive isotopes. Pectin activates the immune system of a human constitution.

Combined accepting of granulated apple pectin with Aronia syrup - a **unique product** -**PEKTARON**, quite recently produced by the Laboratory's manufacturing unit - strengthens favourable biological effect of pectic substances. High content of stable anthocyanins in the berries of Aronia melanocarpa contributes to binding and

excreting of heavy metals and radioactive isotopes. The product has immune stimulating effect and keeps human health in good condition.

#### PHYTEX - NEW SOLUTIONS FOR EXTRACTION OF BOTANICALS

Prof. Lyubomir Boyadzhiev, DSc, Corr. Mem. Institute of Chemical Engineering, Acad. G. Bonchev Str., Bl. 103, 1113 Sofia, Bulgaria

## Application area: Pharmaceutical, cosmetic, and food inductries.

World consumption of herb and spice extracts is continuously growing. Nowadays, more than two-thirds of the pharmaceuticals sold in the world market are completely or partially derived from various plants. Considering this fact and the accumulated for more than 30 years experience in the field of extraction processes, the Institute of Chamical Engineering of Bulgarian Academy of Sciences launched an intensive research program oriented towards development of efficient extraction processes and value-added products. As a result PHYTEX technology was born.

PHYTEX extraction technology is a multipurpose system, designed for extraction of various pharmaceutical substances, flavors, colorants and other valuable components from wild or cultivated botanicals. After the extraction process is completed, retained solvent is removed from the exhausted solid mass. Recovered solvent is reused in the process. The exhausted vegetable mass can be deposited with no harm for the environment or even directly used for feeding the farm livestock. PHYTEX technology can be easily adopted as a water-steam distillation system, suitable for production of natural aromas or esential cils. HMIEX extraction systems are versatile, multipurpose equipment; they can operate as continuous differential extractors (percolators) or in a multi-cycle cross flow regime, both offering complete extraction of the desired component (s). The extractors can be easily transformed into watersteam distillation devices, suitable for production of natural aromas or essential oils. They can perform, in a consecutive order, both processes with the same equipment.

PHYTEX extractors operate at atmospheric or reduced pressure. The latter mode is applied in case of thermo-sensitive products. Choosing the appropriate vacuum level, one can remove most of the solvents residue from the enriched extract, operating in the range of 30 to 50° C.

Using PHYTEX equipment, a large number of extracts, oleoresins and essential oils are produced from common or less known botanicals as:

Acacia, basil, bearberry, black elder, buckthom, caraway, celery, chamomile, common balm, dandelion, deadly nightshade, dill weed, eyebright, fernel, garlic, geranium, ginger, hawthorn, hops, hemlock, horsechestnut, horse-radish, hyssop, juniper berry, marigold, mustard, motherwort, nettle, nutmeg, parsley, pepper mint, plantain, red paprika, periwinkle, puncture vine, oregano, osier, savory, St. John's wort, tumeric, valerian, wild letture, wild thyme, etc.

## TRANSPLANTATION OF ANIMAL EMBRYOS IN BULGARIA

Assoc. Prof. D. Kacheva, PhD

Institute of Biology and Immunology of Reproduction "Acad. K. Bratanov" 1113 Sofia, 73 Tsarigradsko shosse Blvd.

Application areas: Reproduction biotechnologies in animals.

The investigations in the field of obtaining, storage and transplantation of preimplantation embryos and manipulations with ova and embryos in vitro are part of the cellular and genetic engineering. These investigations in the reproductive biotechnologies started at the beginning of the 80's of the last century. In 1974 initial studying started and already existed researches were enlarged on the initiative of the CMEA (Council for Mutual Economic Assistance) in some scientific institutes of all ex-socialist countries. According to Academician Bratanov's proposal the preparations started for organizing of the staff in the Institute of Biology and Immunology of Reproduction for the work in this field. In 1977 Assoc. Prof. Dr K. Vlahov, PhD; Assoc. Prof. Dr D. Kacheva, PhD (1978), later Assoc. Prof. Dr M. Petrov, PhD, research associate Dr Hr. Karaivanov, PhD (1980), Assoc. Prof. Dr E. Sapundjiev, PhD were included in the membership of the section. The first experiments for the transplantation of sheep and cow embryos were prepared and implemented with financial support of the Agricultural Academy and personally of its President Acad. Tz. Hinkovski. In July 1979 in the agricultural experimental station of the Institute of Biology and Immunology of Reproduction - Sofia together with scientists from Poland transplantation of fresh sheep embryos and deep frozen sheep embryos was realized. The embryos were imported from Poland. In December lambs were born from fresh and frozen embryos. These were the first animals-transplants that were born in our country. During the same year the team realized successful transplantation of sheep embryos in the Institute of Upland Stockbreeding and Agriculture (IUSA), Troyan, Bulgaria. By this action they started a long useful collaboration, almost a quarter of a century, between IBIR and IUSA. Successful transplantation of cow embryos was realized in productive conditions

in the village of R. Konare. During 1982 the first calf was born from transplantation. During the same year the transfer of frozen cattle embryos from which the calves transplants were born was realized for the first time. Frozen calf embryos were transported from Czechoslovakia within the frames of mutual scientific collaboration with scientists from the Institute of Physiology and Genetics of Animals of the Czech Academy of Sciences.

Successful experimental export of Bulgarian frozen sheep embryos was materialized in the Institute of Genetics and Breeding of Animals at the Polish Academy of Sciences, as well as in the Agrarian Faculty of the University in Zagreb, where the first lambs from frozen embryos in ex-Yugoslavia were born. The successful interbred transfer of embryos from threatened with extinction ancient native Bulgarian animal breeds were realized during next years. Experimental cryo bank was also created for frozen embryos from aborigine national breeds of sheep, goats and cattle to store their genetic fund.

We used the embryo transfer also to obtain clinically healthy serum negative generation from sheep, which were ill from viral pulmonary adenocarcinoma through interbreed transfer of embryos. Experimental laboratory model was developed on pulmonary adenocarcinoma in mice and the genesis of cancer of this zooanthroponosis was investigated.

Experimentally, after transfer of allogen embryos between inbred mice which have difference of H-2 locus Major Histocompatibility Complex / MHC / the immunity reaction of the recipients was modified and accelerated rejection of the viral leukoma tumor was achieved, and vice versa, increase of metilholanthrene sarcoma growth was reached.

During all these years there was scientific improvement of the elements of the embryo transfer technology in relation to induction of superovu. . . . . . .

lation, obtaining, storage and transfer of sheep embryos. Identical twins were obtained by microsurgical separation of embryos, experiments for obtaining animal chimeras, etc.

Successful results and actuality of the team's elaborations in transfer of buffalo embryos that started as early as in1984 are worth noting. In connection with the appearance of the "mad cow" disease in Europe and the buffalo's resistance to this disease it became the animal species with economic and scientific priority. The obtained by our team buffalo calf - the first transplant in Europe and the second in the world - provoked the world interest and our scientific publication on this topic was cited over 170 times by foreign scientists. The results of investigations were reported at World Congresses in Cairo, Varna and Delhi. They were published in prestigious scientific journals. Joint experiments with scientists from the USA were realised in our country. Lectures and practical demonstrations were delivered at the international course at the Institute of Buffalo Breeding in Shumen during 1988. The first buffalo calf-transplant in India was born in the result of successful participation of the researchers from our section in demonstrations of the embryo transfer.

The Bulgarian team as one the leaders in the field of animal reproduction was invited by the Egyptian Academy of Sciences for a partner in scientific project for buffalo embryo transplantation. Leaders of the project from Bulgarian part were Assoc. Prof. Dr D. Kacheva, and from Egyptian part - Prof. O. Kandil. As a result of common experiments in 2004 the first buffalo calf transplant was obtained in Egypt. The successful results were reported in Egyptian press. The Veterinary Faculty in Cairo awarded Assoc. Prof. Dr D. Kacheva a prize. She is a reviewer of the dissertation thesis on buffalo embryo transfer for PhD degree in Cairo University during 2005. The new pregnant buffalo recipients were obtained from realized mutual scientific experiments the same year. Bulgarian-Egyptian researches have been extended with new scientific problems of reproduction of other domestic animals as well. This is a tradition of the section. At the Veterinary Institute in Skopje our section gave scientific methodical and practical help in joint projects and experiments in Macedonia and Bulgaria. The section still maintains professional relations with Macedonian scientific institutions.

In the course of more than ten years research assistants from the section visited several times scientific institutes for joint courses and scientific experiments in Czech Republic, Poland, Germany, Romania, USSR and Hungary. This was in the frame of the temporal research team for embryo transplantation of CMEA (Council for Mutual Economic Assistance). The opverments of these countries financed these experiments. As members of the international team Assoc. Prof. Dr K. Vlahov, Assoc. Prof. Dr D. Kacheva, and Assoc. Prof. Dr M. Petrov were awarded a prize from the German and Czech Academies of Sciences for successful scientific work. Part of scientific publications of our section is common and they are results from scientific-technical collaboration between institutes in Italy, Egypt, India, N. Korea, Mongolia, Macedonia, Croatia and us.

Embryo transplantation with many scientific problems, veterinary aspects and economical resources for stockbreeding are topical and important for the world. Our team at the Institute of Biology and Immunology of Reproduction - BAS is the only one that works in this field in Bulgaria and deserves administrative and financial support.



## EQUAL IN EUROPEAN RESEARCH AREA

#### BULGARIAN VIPs:

#### Acad. ATANAS ATANASSOV, DSc



Dr. Biology (1977); DSc. Agronomy (1989); Corresponding member (1997) and member (2003) of the Bulgarian Academy of Sciences; Member of Agricultural Academy of the Ukraine (1993) and Agricultural Academy of Russia (2003). Doctor Honoris Causa of the University of Agricultural Sciences and Veterinary Medicine, Kluj-Napoca, Romania (2001). Director of the Central Laboratory of Genetic Engineering (since 1985), renamed Institute of Genetic Engineering (1989) and AgroBioInstitute (2000) - Center of Excellence in Plant Biotechnology (2000), National Center for Agricultural Sciences, Sofia, Bulgaria; Member of the Board of Governors of Agricultural Academy; Professor and Director of the Bulgarian part of the International Center on Plant Science, De Montford University, Leicester, UK (1994); National coordinator and a representative of the Bulgarian Affiliated Center and Member of the Board of

Governors of the International Center of Genetic Engineering and Biotechnology (I.C.G.E.B.), Trieste, Italy; Member of Steering Committee of UNEP Multi-year programme for Capacity Building and Regional Co-operation on Safety in Biotechnology in Central and Eastern Europe (CEE) (1995) and a FACE Program of EC (1995); Chairman of Steering Committee on Biosafety for CEE (2002 - 2004); Executive Secretary of Bulgarian Council for Biosafety Use of Genetically Modified Higher Plants, Ministry of Agriculture and Forestry (1996 - 2005); National coordinator for Plant Biotechnology in Bulgaria. Coordinator of Plant Biotechnology for Balkan countries (1995); Expert to FAO's Plant Biotechnology Program, Rome, Italy and IAFA, Vienna, Austria; National Correspondent of the International Plant Tissue Culture Association, European Association for Research and Plant Breeding - EUCARPIA, International Society for Molecular Plant-Microbe Interaction (IS-MPMI), Japan Society of Plant Physiology, ESNA - European Society for New Methods in Agricultural Research; Chairman of the National Committee of Bioethics, Ministry of Education and Sciences, Bulgaria (2002); Chairman of Bulgarian Association of Seed and Planting Material (since 2001); President of AqroBioTech Park (since 2003); President and Chairman of the Board of Directors of the Black Sea Biotechnology Association (since 2004).

Acad. A. Atanassov specializes in tissue culture at the Institute of Plant Physiology, Moscow, USSR (1969, supervisor: R. G. Butenko, one of the most famous scientists in the field of plant cell and tissue cultures). He passed training on pollen and protoplasts regeneration (1975) in the laboratories . . . . . . . . . . . . . . . .

of Dr. Kollet Nitch, ONRS, Jiff-sur-Ivette, and Dr. Jean-Pierre Boulrein, INRA, Versaille, France, on the problems of somatic hybridization (1991) in the laboratory of Dr. Denesh Dudits, Szeged, Hungary and regeneration and transformation in alfalfa in the laboratory of Dr. Wilf Keller, Canada (1991). He obtained practical experience in molecular genetics (1987) at the University of Gent, Belgium and at the Institute of Plant Science, Giff-sur-Ivette, France. These trainings helped him to go deeply into the world experience on plant cell and tissue culture and gave him and his colleagues possibility to start a successful work on the current problems of plant genetics, tissue culture, cell and genetic engineering for certain number of important agricultural crops.

Acad. A. Atanassov started his scientific carrier in 1967 as a Research Assistant at the Tissue Culture Laboratory of the Institute of Sugar beet near Shumen, Bulgaria. This was a pioneer plant biotechnology laboratory in Bulgaria. In the beginning his work was oriented towards organogenesis, androgenesis and vegetative propagation in sugarbeet tissue cultures. The most important and perspective results important for sugarbeet genetics and breeding were results related to regeneration and clonal micropropagation.

From 01.10.1974 till 1982 he had a position of a researcher at the Laboratory of Tissue Culture, Institute of Genetics, Bulgarian Academy of Sciences. On 23.11.1982 he was elected a senior scientist. At that time the field of his investigations was enlarged by tissue cultures from tobacco, *Datura innokia*, triticale, sunflower, alfalfa, pea and several pharmaceutical plants. In some of them (like alfalfa, sunflower etc.) original data have been obtained. As a result, the first biotechnological Bulgarian varieties - Biopreslavna (tobacco) and Inka (*Datura innokia*) have been created.

Further, the main part of his activities was directed towards plant protoplasts problems for development of techniques for somatic hybridization and genetics transformation.

On 15.04.1985 he was nominated for the Director of the Central Laboratory of Genetic Engineering (CLGE), renamed in 1988 as Institute of Genetic Engineering (IGE) and now - since 2000 - AgroBioInstitute (ABI), belonging to the National Center of Agricultural Sciences, Sofia, Bulgaria.

During the last 15 years AgroBioInstitute succeeded to reach the status of a basic scientific, coordinating and methodological center for plant biotechnology in the country. The obtained theoretical and practical results on regeneration, gene transfer, cloning of important genes, related to viral resistance, cytoplasmic male sterility in important plant crops as alfalfa, tobacco, barley, sunflower, camation, sugarbeet as well as application of molecular markers in *Vitis* sp., oil rose, barley, wheat, maize etc. and clonal propagation *in vitro* made the institute a well known center abroad. Due to the entire work in 2000 EC nominated AgroBioInstitute – Kostinbrod for a Center of Excellence in Plant Biotechnology.

Acad. A. Atanassov has published 220 publications in refereed journals, above 150 reports and 150 posters presented at conferences and symposia, 9 chapters of books, 2 manuals, and 4 books.

#### Professor ANGEL SIMEONOV GALABOV, MD, DSc, Corr. Mem.

Born in 1939 in Sredetz, Stara Zagora district, Bulgaria. Graduated in Medicine at the Medical University of Sofia (1962). Defended his PhD thesis in Biology (1968) [Study of the Inhibiting Activity of N', N'-anhydrobis (b-hydroxyethyl) on the Replication of *Paramyxovirus parainfluenzae* 1 (Sendai)] and his DSc in Virology (1978) [N, N'-Disubstituted Thicurea and Abitylguanid - Specific Viral Inhibitors]. Academic ranks: Associate Professor (1974), Professor (1988). Postdoctoral Fellowship at the Institut Pasteur, Paris with the Nobel Prize winner Andre Lvoff (1968/69) and with Acad. Vadim Agol (Moscow State University) (1975).

Angel S. Galabov is currently the most distinguished virology scientist in Bulgaria and is widely acclaimed worldwide. His work and publications comprise 162 scientific studies, including a monograph and a monograph section (published by Academic Press). 95 of his works have been published in renowned international editions. He has been cited in over 550 references.

Prof. Galabov's main scientific contributions are connected with antiviral substances - experimental chemotherapy of viral infections and biological response modifiers.

In close collaboration with almost all leading groups of Bulgarian chemists-synthetics and chemistry specialists on chemistry of natural substances, Angel Galabov has discovered and identified a number of highly active viral replication inhibitors. These include:

> N,N'-disubstituted thiourea, active against enteroviruses and other picomaviruses, among which the compound PTU-23 is considered prominent with its antienteroviral effect and in vivo activity and is regarded as a "classic" enteroviral inhibitor;

> derivatives of tetrahydro-2[1H]pyrimidinone and other cyclic urea, effective against influenza, toga- and flaviviruses. The most effective among them is mopyridone, which is highly active in experimental infections with influenza A and B viruses and has a synergistic effect in combination with rimantadine. Its effect upon alphaviruses (which cause several diseases spread by mosquitoes in the tropics) determines it as the most effective



antialphavirus substance, described in scientific literature;

benzodioxan derivatives (BMM-8, BMM-10) - alpha- and flavivirus manifesting a protective invivo effect;

> aporphinoidal alkaloid oxoglaucine-with a pronounced inhibitory effect upon enteroviruses replication.

These researches were successful due to the methodology developed by Angel S. Galabov - in creating new methods and testing-patterns of antiviral substances, including a screening system for viral replication inhibitors.

Prof. Galabov reveals the molecular mechanism of antiviral activity of the most perspective new antivirals proved by him and determines the target of the virus inhibitors-ligands of viral proteins: for thiourea PIU-23 - the protein in enteroviruses regulating the replication cycle, for tetrahydro-2 [1H]pyrimidinone mopyridone - the matrix protein ML of influenza A virus, for biguanide abityilguanide - a capsid protein of adenoviruses.

In his investigations together with chemists from Bulgaria and the Ukraine, Prof. Galabov succeeded in clarifying the chemical structureantiviral activity relationship in active classes of compounds (N, N' - disubstituted thioureas, tetrahydro-2[1H]pyrimidinones), which serve as a base for directed synthesis of new highly active compounds.

Angel S. Galabov has been working in the field of drug-resistance, stressing mainly on en-

teroviral inhibitors - which are of utmost importance in human pathology as etiological agents of neuroinfections, diabetes, heart diseases, etc. These viruses are characterized by a high mutation rate with a rapid selection of resistant mutants and initiation of different illnesses by one and the same enteroviral species. Prof. Galabov's contribution to clarifying this phenomenon is of greatest importance, and as a means of overcoming it as a serious problem and achieving effective chemotherapy, he has found out, together with his associates, a series of synergic combinations of enteroviral inhibitors.

His is the credit for creating and developing the first antiadenoviral chemotherapeutic agent ADENOSTATINâ (based on abitylguanide), effective in the treatment of epidemic keratoconjunctivitis. The medicine is being manufactured and is also employed in ophthalmologic practice.

It is the first Bulgarian antiviral chemotherapeutic agent.

His another achievement is cycloridine - the first chemotherapeutic agent that is effective against the flaviviral infectious mucous disease viral diarrhoea in calves, which causes great damage to cattle breeding. The agent is also effective against lethal infection in vivo caused by the tickborn encephalitis virus.

Angel S. Galabov has also developed an agent for prophylactics against nuclear polyedrosis in silkworms, which causes serious damage to silkworm breeding.

An important part in his research is allotted to the study of interferon, its inducers and other biological response modifiers. Working on the problem phylogenesis - interferonogenesis, he proved and characterized the interferon system in reptiles.

As a result of systematic investigation Prof. Galabov determined the structure-carrier of interferon-inducing properties of endotoxines in enterobacteria. Based on these results, he has been working on their detoxication and has succeeded in obtaining the utmost effective and harmless interferon inducer based on this natural product. Later he characterized a wide range of lowmolecular weight interferon inducers as inhibitors of the enzyme phosphodiesterase of the cyclic adenosine monophosphate. This serves as a base for directed selection of new highly effective interferon inducers (dipyridamol) used in prophylactics of epidemic influenza, herpes simplex, etc. Òhis initiates further research made by a number of scientific groups world-wide. In collaboration with associates from the Institute of Molecular Biology of the Bulgarian Academy of Sciences a technology for the production of lymphoblastoid interferon has been developed and approved for clinical testing.

Another part of Prof. Galabov's achievement is his research into the effect of substances, which neutralize the oxidative stress in influenza virus infections – antioxidants of natural (vitamins E and C) and synthetic (Yonol) origin and the chemotherapeutic agent rimantadime. Angel S. Galabov has proved the protective effect of anti-prostaglandine indomethacine in experimental herpes infections.

Of great importance of his research on virucidal agents is determining the antiviral range of Manorapidâ Synergy - a highly effective virucidal composition applied in clinical practice as a hands disinfectant.

Prof. Galabov has contributed to the etiological role of neurotropic viruses in acquired diabetes, to the participation of hantaviruses in the pathogenesis of the Balkan endemic nephropathy (a lethal disease affecting Northwestern Bulgaria and some regions of neighboring Balkan countries) and to the role of human papillomaviruses as carcinogenic in the pre-cancer state and of in the uterus cervix cancer in Bulgarian population.

Prof. Galabov's activity as an inventor and author of 39 inventions (three of them patented abroad) should also be emphasized. His name is included in the Golden Book of Investigators of the National Patent Office (2003) for his inventions in the field of antiviral agents. He has also received a number of other distinctions for his inventions.

Angel S. Galabov is in charge of numerous scientific projects (16), financially supported by programmes of the European Union, other foreign scientific foundations, the Bulgarian National Fund for Scientific Research, etc. He has acted as coordinator of the topic "Antiviral Substances" in the multilateral cooperation of the Academies of Sciences of the countries of Central and Eastern Europe. Another 16 contracted projects have been concluded with companies for testing substances for antiviral activity and virucidal activity by employing the screening systems developed by him.

Angel S. Galabov has read more than 140 reports at international scientific forums, 24 of them being plenary or following an invitation. He has presided at meetings and sections at 26 international scientific events. He was invited to conduct seminars in 17 foreign universities and scientific institutes.

Prof. Galabov is a member of the international editorial boards of the following scientific journals: "Acta virologica" (the first European specialized journal on virology), "Future Microbiology", "Problems in Russian Virology", "Balkan Journal of Medical Genetics", "Interferon & Biotechnology", two foreign national scientific journals, and the Bulgarian "Biotechnology and Biotechnological Equipment" and "Bulgarian Medicine".

In his area of research he has been supervisor of 19 PhD students, 11 of them having defended their theses, and of a number of other young specialists from Bulgaria and abroad. More that 10 PhD theses of specialists from other Bulgarian and foreign scientific groups are based on his research.

Angel S. Galabov has founded two laboratories specialized in antiviral substances – at the Medical Academy, Sofia, and at the Institute of Microbiology (BAS). The importance of these laboratories is highly appreciated by scientists all over the world and they are acknowledged as leading centers for research of antiviral substances in Central and Eastern Europe, and one of the most significant centres in Europe in general.

This activity of vast scope and high quality characterizes the creation of a Bulgarian school connected with the research of antiviral substances.

Angel S. Galabov has been a longstanding expert on viral infections (since 1983) of the World Health Organization.

His scientific research correlates with a wide range of activities, which involve initiating and organizing scientific forums and working in scientific organizations. He initiated and actively took part in the functioning of the first symposium chain on antiviral substances in Europe (since 1974). He also initiated the foundation of the Balkan Society for Microbiology (BSM) in 1998 and was its main organizer and first President (1999-2001). Currently he is a member of the Board of BSM and Chairman of the Programme Committee of BSM. Angel S. Galabov is initiator of the most significant activity of BSM - the Microbiologia Balkanica forums, which take place every two years and have great success in integrating the efforts of all microbiology scientists in Southeastern Europe. He had two mandates as a Member of the International Research Council of the International Society for Antiviral Research, ISAR (1992-96) and won high approval for his work, including his effort in incorporating in ISAR scientists from Central and Eastern Europe. He was also a member of the Permanent Organizational Committee of the International Antiviral Symposia; he is also a member of six other international scientific organizations.

An active member of the Union of Scientists of Bulgaria, since 2001 Prof. Galabov is the President of the Bulgarian Society for Microbiology and a member of the Board of the Union of Scientists of Bulgaria. He represents Bulgaria in the international scientific organizations FEMS (Federation of European Microbiological Societies) and TUMS (International Union of Microbiological Societies). He is an organizer of many scientific activities in microbiology and virology in Bulgaria, the most important and perfectly organized being the 10th Congress of Microbiologists in Bulgaria (2002) and "Virology Days in Bulgaria" (2004). Angel S. Galabov is one of the co-founders of the Bulgarian National Academy of Medicine (1995) and a member of its Academic Board.

He has been a long-standing member of the Specialized Scientific Council of Virology and actually of Microbiology, and Virology and Immunology with the High Attestation Commission, a member of scientific boards of a number of institutes, member of the expert council at the Ministry of Health in Bulgaria, President of the National Sub-committee for policyelitis eradication, and of the Committee of Biological Defense, etc.

He has been involved to a great extent as a reviewer in scientific boards of the High Attestation Commission, as an expert in the Bulgarian National Fund for Scientific Research, etc.

Prof. Galabov is a member of the General Assembly of the Bulgarian Academy of Sciences (BAS) since its foundation in 1993 and member of the Council for National Security and Safety of the Executive Council of BAS, and other expert committees in BAS.

Since 1995 he is the Director (third mandate since 2003) of the Institute of Microbiology (BAS). He has contributed to a very great extent to the institute's recognition as a national research centre in microbiology and as a competent scientific institution in Southeastern Europe. On his initiative the Institute of Microbiology was associated to the Institut Pasteur, Paris (26.06.2004). The institute became the eighth European institute in this family of associated members. The Institute of Microbiology of the Bulgarian Academy of Sciences has thus attained high recognition for its level of scientific research and intensive activity. Angel S. Galabov has become a regular member of the Board of Directors of the Institut Pasteur and associated institutes. Meanwhile he was also chosen as coordinator for one of the research directions of the institutes in Europe.

In the crucial environment of the recent economic realities Prof. Galabov's aim was to incorporate the Institute of Microbiology in the international market of scientific products - by strictly observing project financing as a base for scientific and research work, by developing international scientific cooperation, and the Institute has been rated as being among the most active regarding programmes of the EU and NATO (an award given to Prof. Galabov by the Ministry of Education and Science). The increasing number of publications in international scientific journals should also be mentioned, as well as the excellent personnel policy with special attention paid to young scientists, the increase in the Institute's revenue (first place among the biological institutes of BAS), and the developing teaching activity. A very successful tendency is the international courses for young specialists from Southeastern Europe, which are organized at the Institut Pasteur Atelier in the Institute of Microbiology.

Angel S. Galabov has a long experience as a teacher in virology: for post-graduate qualification of students in the former Institute for Postgraduate Training of Physicians and the Medical Academy, at the Faculty of Veterinary Medicine of the Forestry and Technology University in Sofia (founder of the Department of Microbiology and endorsing virology as a separate discipline, creator of the teaching syllabus and of the virology course), at the Faculty of Biology at Sofia University as a teacher in specialized courses in medical virology and antiviral agents (master degree courses and as diploma works supervisor), as initiator and founder of the first course of lectures in virology for students of medicine (currently an optional course) in the Medical Faculty of the Medical University, Sofia, lecturer of pre-qualification courses for pharmaceutics at the Faculty of Pharmaceutics of the Medical University, Sofia.

Prof. Galabov is also famous for his active work connected with popularizing scientific knowledge via printed works and electronic media (approximately 250 interviews and publications, including a book and a radio lecture course) - on the newest achievements in the field of virology, on current viral infections and how to fight them. He is also involved in popularizing the achievements of his institute and of the Bulgarian Academy of Sciences.

In 2004 Angel S. Galabov was elected a Corresponding Member of the Bulgarian Academy of Sciences.

In 2005 the Romanian Academy of Medical Sciences elected him a "Member of Honour" for his contribution as a scientist in virology and for his achievements related to scientific cooperation in the field of microbiology in the region of Southeastern Europe.

The Russian Academy of Medical Sciences awarded him with the Medal "D. I. Ivanovsky" for his contribution in the development of virology and international cooperation in this scientific field.
## Associate Professor IVO KREMENSKY, MD, PhD

Assoc. Prof. Ivo Kremensky is one of the most eminent Bulgarian scientists in the field of Biochemistry and Molecular Genetics of Human Diseases. As an expert in genetic screening programs, he has comprehensive overview on the hereditary pathology in the Bulgarian population, and knowledge of the health care infrastructure of the country. He is well recognized in all aspects of organization and implementation of large-scale diagnostics and prevention programs based on genetic analysis. Assoc. Prof. Kremensky is actively involved in the legislation and policy-making process in the field of healthcare in Bulgaria and other issues, related to genetic testing.

Assoc. Prof. Kremensky has made major contributions to the introduction of two national mass screening programs of newborns for phenylketonuria and galactosemia (since 1977), to Biochemical screening for Down syndrome and neural tube defects in the second trimester of pregnancy (since 2000), and the DNA prenatal diagnosis of aneuploidias (since 2001). One of his major achievements is the design and implementation of the first two National Programs for Diagnosis and Prevention of Inherited Disorders and Congenital Anomalies in Bulgaria (2000-2005 and 2006-2010). He has been principal investigator or collaborator in 17 genetic studies, 12 of which were international collaborations.

Assoc. Prof. Kremensky, together with Prof. Luba Kalaydjieva, founded in 1977 the Laboratory of Molecular Pathology, Sofia Medical University and is currently the Head of Laboratories at the University Hospital of Obstetrics and Gynaecology "Maichin Dom". The Laboratory of Molecular Pathology is the major diagnostic, research and training centre for biochemical and molecular genetics in Bulgaria. The research team of Assoc. Prof. Kremensky is internationally recognized in the field of inborn errors of metabolism, genetics of neuromuscular and psychiatric disorders and population genetics. The Medical Faculty, Sofia Medical University, awarded Assoc. Prof. Kremensky the "Acad. Dr. D. Orahovatz" prize for research excellence in 2001.

Over the past 10 years Assoc. Prof. Kremensky published more than 130 papers in international peer reviewed journals, including *Nature Genetics* and the *American Journal of Human Genetics*. He has a long teaching carrier at Sofia Medical University and is involved in the undergraduate and postgraduate medical and biology education with lectures and tutorials in molecular pathology. Assoc. Prof. Kremensky is the author of the first fulltime course in Molecular Medicine at Sofia Medical University. He has supervised more than 24 MSc and 15 PhD students.

Assoc. Prof. Kremensky is a member of the governing administrative and scientific body of the Medical Faculty, Sofia Medical University, the National Council on Genetics at the Ministry of Health, chairman of the Scientific Panel on Medicine and member of the Scientific Panel on Genomics at the Ministry of Education and Science. He is a member of the Panel on Genetics and Molecular Biology, Biochemistry and Biophysics of the Higher Accreditation Commission of Bulgaria.

Assoc. Prof. Kremensky is an expert member of the International Federation of Clinical Chemistry and participates in a number of prestigious international organizations, as International Society for Neonatal Screening, International Society of Inherited Metabolic Diseases, American Society of Human Genetics, International Working group on Nanotechnology and European Down's Syndrome Screening Group.

## AWARDS

## SCIENTISTS AWARDED IN THE CONTEST FOR HIGH SCIENTIFIC ACHIEVEMENTS IN 2005

## A DIPLOMA FOR HIGH SCIENTIFIC ACHIEVE-MENTS AND CASH PRIZE

#### A. YOUNG SCIENTISTS

• Chief assistant MARIA PETKOVA TOKMAKOVA from the Clinic of Cardiology at the University Multiprofile Hospital for Active Treatment (UMHAT) of Medical University in Plovdiv for three publications in prestigious medical journals and participation in a monograph in the field of chronical cardiovascular insufficiency.

The main contributions of the works are connected with risk evaluation at patients with cardiovascular insufficiency and possibilities for influencing the prognosis. These accents are among the present day problems of modern cardiovascular practice.

• Senior research associate NIKOLAI MARINOV NIKOLOV from the Institute of Mathematics and Informatics at the Bulgarian Academy of Sciences (BAS) for 12 publications in the field of multidimensional complex analysis - 11 in foreign journals and 1 in the *Reports of the Bulgarian Academy of Sciences* during the last three years.

The investigations are connected with the border behavior of invariant metrics. This is a very important topic having application in problems of complex analysis for continuation of holomorphic images. The theorem on localization of the kernel and the Bergman metric round the holomorphic peak point is proved.

#### B. SCIENTISTS OVER 35 YEARS OLD

• Prof. VLADIMIR DAMGOV from the Space Research Institute at BAS for the monograph "Nonlinear and Parametric Phenomena: Theory and Applications in Radiophysical and Mechanical Systems". World Scientific: New Jersey, London, Singapore, 2004.

Capital work in the field of nonlinear phenomena with application in microelectromechanical systems and nanotechnologies. Original and important scientific and applied contributions to theoretical calculations and experimental research of parametric, nonlinear and nonlinear-technical oscillating systems are reflected in the work.

• Senior research associate DORA KARAGYOZOVA from the Institute of Mechanics at BAS for 11 scientific publications abroad in the field of shell deformation at intensive dynamic load causing great plastic deformations as well as characterization of cell materials at great plastic deformations.

Methods for investigation of complex rod systems with cell hexagonal structure at biaxial power action are created and different states at pipes with ring-shaped and orthogonal vertical section are defined. The conclusions made can be applied at designing tubular mechanisms and constructions from aluminium and steel alloys at impulse excitations.

 Senior research associate I grade, DSc. ILIYA DIMITROV HRISTOV from the Institute for Soil Science and Agroecology "N. Pushkarov" for his monograph "Estimation of Water Status of Agroecosystems and Formation of Water Reserves in Soils". Publishing House "Publish SaiAT-ECO", Sofia, 2004.

The author for the first time in this country and abroad presents a new scientific basis for evaluation of water status of the system "soil – agroculture – ground layer of the atmosphere" for the whole vegetation period and its efficiency. A new integral index and method for determining it are introduced for energy levels of soil moisture.

• Senior research associate NADYA MLADENOVA ANTONOVA from the Institute of Mechanics and Biomechanics at BAS for 8 scientific publications in prestigious international editions in the field of haemo- and biorheology, as well as for investigation of dependences between surface forces by means of experimental observation of parameters of thin liquid films from pulmonary surfactants.

Important haemorheological dependences are found with patients with cerebrovascular disorders

of ischemical origin and hyperviscosity syndrome, as well as the impact of high-molecular polymeric additives upon haemorheological and haemodynamical parameters with animals.

 Associate professor HRISTO SLAVOV
KYUCHUKOV from Veliko Tarnovo University "St.
Cyrill and Methodius" for his two monographs "Methods of Teaching Bulgarian Language in Elementary School in Conditions of Bilinguism", "Prosveta" Publishing House, 2004 and "Written Speech in Bulgarian of Romany Schoolchildren in III – V Grades", Balkan Foundation "Diversity", 2002.

The problems treated in the book are very topical not only for our country, but also for the rest of the European countries. In the light of integration processes of ethnic minorities, preserving their ethnic culture and specific features, the offered methods of teaching Bulgarian language and developing writing skills have exceptional applied value for our system of education.

## HONORARY DIPLOMA FOR ACHIEVEMENTS IN SCIENCE

#### A. DOCTORANDS

• Dr. VENTSISLAVA YANKOVA PETROVA - part-time assistant at Biological faculty of Sofia University "St. Kliment Ohridski" for defended in 2004 dissertation "Investigation of Mechanisms for Detoxication of Reactive Oxygen Species in Mitochondries of Yeast" and three articles in foreign publications connected with the dissertation.

On the basis of conducted original experiments a number of dependences are proved in the dissertation and a new model for *in vivo* detoxication of active oxygen generated in cell mitochondries is offered.

 Research associate KLIMENT BORISOV
PETROV from the Institute of Molecular Biology at BAS for defended in 2004 dissertation "Expression of Fragments of Antibodies and Strategies for Their Stabilization" and two articles in foreign journals.

This is an important and topical problem in the field of albumen engineering. Systems for expression of catalytic antibody, for determining optimal conditions for cultivation, creation of a new pseudo-fragment and other vanguard approaches applicable for solving problems in the field of molecular immunology are developed.

#### B. SCIENTISTS OVER 35 YEARS OLD

• Associate professor **ALEXANDAR OMARCHEVSKI** from the Faculty of Theology at Sofia University "St. Kliment Onridski" for his works "Religious Policy of the Emperor Justinian I (527 – 565)". Publishing House of Sofia University "St. Kliment Ohridski", 2004 and "In Search of Truth" (Publication of the Faculty of Theology, 2003).

The works investigate problems not studied in detail in the national theology. A thorough analysis of very contradictory in their contents conclusions about Justinian I and other persons as well as of new and up to now not investigated by Bulgarian science facts and documents is done.

 Associate professor DIMITAR DIMITROV
POPOV from Shumen University "Bishop K.
Preslavski" for the monograph " Phonostylistics of the Discourse", University Publishing House, Shumen, 2004.

This is the first monographic research on phonostylistics in Bulgarian linguistics and the first serious attempt to bring phonostylistics forward as an independent subject within the sphere of linguistics. The work contains serious theoretical treatments connected with the sound qualities, pronunciation and pronunciational qualities of Bulgarian speech.

Associate professor NIKOLAI STANEV
PRODANOV from Veliko Tarnovo University "St.
Cyrill and Methodius" for his monograph "Problems of Historiographic Textology. On the Material of Bulgarian Historiography of VII -XX Centuries".
"Faber" Publishing House, V. Tarnovo, 2003.

The work fills in the gap in the Bulgarian historiographic research. For the first time the study of the most important historiographic conclusion historically written text - is put on a broad basis.

• Senior research associate **RAYA ZAIMOVA** from the Institute of Balkan Studies at BAS for the monograph "The Way to the "Other" Europe. From French-Ottoman Cultural Intercourse in the XVI-XBVIII Centuries." "Queen MAB" Publishing House, 2004.

The work contributes to clarification of cultural intercourse and acquaintance of separate parts of Europe in the course of formation of modern self-identifications of European nations and getting general idea of the Europeans.

• Professor **STOYAN GEORGIEV DENCHEV**, Dr. of Econ. Sc., rector of the State Institute of Library Studies and Information Technologies, for his monograph "Insecurity, Complexity, and Information: Analysis and Development of Insecure Information Environment"; 'Z. Stoyanov" and "St. Kliment Ohridski" Publishing Houses, 2004. Co-author of the book is Dr. D. Hristozov, professor at the American University in Blagevgrad, but with little participation.

The monograph is a serious study of current topics and has definite scientific contributions, which may have practical application. Theorems and approaches for simplification of complex systems with minimum loss of information are proved. Methods for technical-economical comparison of products on the basis of expert systems are developed, as well as methods for designing of information environment.

## HONORARY DIPLOMAS FOR SCIENTIFIC - APPLIED CONTRIBUTION

#### A. DOCTORANDS

• Research associate **EKATERINA LYUBENOVA MARKOVA** from the Institute of Sociology at BAS for her dissertation "Problems at Optimization of the Excerpt Data Incomplete in Scope" under the guidance of Corr. Member At. Atanasov, defended in 2004.

Serious theoretical violations and practical "distortions" in the used information from excerpt investigations ignoring incompleteness of the obtained data are proved. The work is useful not only for conducting excerpt investigations, but also for systematic social, demographic and business investigations.

## B. SCIENTISTS OVER 35 YEARS OLD

Associate professor MASIS BERDIS
HADZHOLYAN from Varna Free University "Ch.

Hrabar" for the Book "European Constitutional Law", University Publishing House, Varna, 2004.

The work is the first Bulgarian monographic study dedicated to a current problem of modem science. It has essential contribution to the development of the Bulgarian legal culture, theory and practice. The author's comparisons, generalizations and ideas dedicated to organization of constitutional state, citizen, civil society and construction of the state power in the statesmembers of the European Union and Bulgaria are original.

#### AWARDS FOR JOURNALISTS

#### A DIPLOMA AND CASH PRIZE

• VELIANA HRISTOVA, PhD, analyzer-commentator on the problems of science and education in daily newspaper "Duma". From the presented 50 main publications during the period from October 1, 2004 till October 1, 2005 on the problems of science and higher education, 12 articles are directly connected with the activities of the Union of Scientists in Bulgaria (USB).

Mrs. Hristova is very popular among scholars and scientists owing to her deep knowledge of problems of science and higher education, of scientists and university lecturers.

#### A DIPLOMA

• EKATERINA PAVLOVA - chief of the "Cultural Panorama" department in the monthly periodical "Bulgarian Diplomatic Review" for a series of publications reflecting achievements of Bulgarian science and culture and activities of the USB. The periodical is published in 3 languages (Bulgarian, Russian, and English) and is distributed in over 100 countries in the world.

## WINNERS IN THE SECOND ANNUAL COMPETITION "INNOVATIVE BULGARIAN ENTERPRISE OF THE YEAR 2005" ANNOUNCED

The Second National Innovation Forum which ARC Fund organized jointly with the World Bank office in Bulgaria, DG Enterprise of the European Commission, the Ministry of Economy, the Austrian Embassy in Sofia, EPIQ Electronic Assembly and the Center for the Study of Democracy on October 25, 2005 featured the awards ceremony for the Second National Contest recognizing the most Innovative Bulgarian Enterprise of the Year 2005.

The main objectives of this contest were:

 to encourage the innovation activities of Bulgarian enterprises; and

• to promote the companies participating in the contest and the results achieved by them in the field of innovation.

Innovative companies from all sectors of the Bulgarian economy were eligible to participate in the contest.

A company was considered to be innovative if it had developed and marketed new or advanced products (goods or services) and/or processes over the last three years. Innovation was defined in broader terms as application of new approaches or technologies improving the economic performance and competitiveness of the respective company.

The innovation performance of participating companies was evaluated by an expert panel against a set of criteria and data collected on the basis of a special application form - an entry questionnaire that companies participating in the contest completed and submitted to ARC Fund.

#### Evaluation

The ranking of participating companies was performed on the basis of a set of criteria combining quantitative and qualitative indicators, measurable against a scale of 1-to-5.

Evaluation criteria:

1 Technical level of the new products/processes: assessment of the basic technical parameters of the new products/processes developed by the contestant as compared to the respective analogue of new products/processes of competing companies. 2 Turnover: relative share of new products/ processes in the total sales of the company.

3 Exports: relative share of the new products/processes placed on foreign markets in the overall export of the company.

4 Prospects: opportunities for further development of the new products/processes.

5 Intellectual Property Rights protection of the new products/processes: intangible assets acquired (patents, IRR certificates, licenses, etc.)

6 R&D expenses: relative share of R&D expenses in the total amount of revenues.

7 R&D personnel: relative share of employees involved in R&D in the overall personnel (this criterion is only valid for large enterprises).

8 Number of new products/processes: number of new products/processes placed on the market in the last 3 years.

9 Connectivity/Networking: company's linkages/cooperation with universities; scientific, research and engineering organizations, participation in international programs, etc.

10. Level of production and management in the enterprise: implemented certification and quality control systems, preservation of the environment, product safety; utilization of computers, software products, network devices, online services offered, e-commerce, etc.

The ranking of companies per their final evaluation was ultimately thoroughly analyzed by the expert panel.

### Awards

In order to ensure comparability with the accepted EU enterprise categories, two groups of companies have been defined:

innovative small enterprises (of up to 50 employees);

 innovative medium-sized or large enterprises (of over 50 employees).

The awards ceremony took place during the National Innovation Forum on October 25, 2005 at the Sheraton Sofia Hotel Balkan. Mr. Plamen . . . . . . . . . . . . . . . .

Vachkov, Chairman of the newly established Agency for IT and Communications, handed in the awards.

The Daisy Technology Ltd. company was presented with the Award for Innovative Small Enterprise of 2005. The SD ELTA – R company won the contest in the category for mediumsized and large enterprise. They were followed by five other SMEs also recognized as innovative, which received Certificates for Innovative Enterprise of the Year 2005: Trapen SP, Coattex Ltd., Antipodes Ltd., Orgachim Co. and Optix Co.

The Daisy Technology Ltd. was ranked first for the following new products it had introduced to the Bulgarian market: mp3 players, Digital Multimedia Cameras. Trapen SP participated in the contest with its microprocessor system for automation and optimization of different kinds of mills for grinding of cement, raw materials, ores, etc., in the cement, dressing and power industries and a number of related branches. Coattex Ltd. is specialized in the field of technologies and techniques for powder polymer coatings, metallic coatings, enameling and talcking and was nominated for its product for electrostatic powder coating machine APEN® -30 G1(2). Antipodes Ltd. works in the field of system architecture and software design, web

and database development, application and system development for a variety of hardware/ software platforms and complex solutions. The company participated in the contest with the following products: a system for visual modeling and automation of business procedures in the Workflow.Cubes company, and with an innovative software solution that provides professionals and amateurs in the field of interior design with the opportunity to prepare proficient interior solutions - 3D Spacer.

SD ELTA - R was ranked first in this category with its product for development of digital telecommunication network in lowly populated regions. **ELTA R** is a company with experience in development, manufacture, engineering and trade in products in the communication industry. Orgachim Ltd. was awarded for the DEKO professional products for construction and interior design. Optix Co. operates in the field of designing, manufacturing and testing quality of optical components and systems, encompassing the capabilities required to undertake opto-mechanical and opto-electronic assemblies and devices meeting the needs of the medicine, military and commercial markets. The company participated in the contest with 13 innovative products.

## RECENT PUBLICATIONS OF BULGARIAN SCIENTISTS

### ARTICLES

Title:	Prospects for Biological Control of Teasels, Dipsacus Spp., a New Target in the United States.
Authors:	Rector, Brian G <sup>1</sup> <u>brector@ars-ebcl.org;</u> Harizanova, Vili², Sforza, René¹; Widmer, Tim¹; Wiedenmann, Robert N <sup>.3</sup>
Source:	Biological Control; Vol. 36, 1 (2006 Jan.), 1-14
Author Affiliations:	<sup>1</sup> USDA-ARS, European Biological Control Laboratory, Montpellier, France; <sup>2</sup> Department of Entomology, Agricultural University, 12 Mendeleev Str., 4000 Plovdiv, Bulgaria; <sup>3</sup> Department of Entomology, University of Arkansas, Fayetteville, AR 72701, USA
ISSN:	1049-9644
Title:	Nonlinear Robust Temperature-Humidity Control in Livestock Buildings.
Authors:	Soldatos, A.G. <sup>1</sup> , <u>asoldat@cc.ece.ntua.grArvanitis;</u> K.G. <sup>2</sup> <u>karvan@aua.gr</u> ; Daskalov, P.I. <sup>3</sup> <u>daskalov@ru.acad.bg</u> ;Pasgianos, G.D. <sup>1</sup> <u>pasgianos@racqu.com</u> ; Sigrimis, N.A. <sup>2</sup> n.sigrimis@computer.org
Source:	Computers & Electronics in Agriculture; Vol. 49, 3 (2005 Dec), 357-376. <sup>1</sup> National Technical University of Athens, Department of Electrical and Computer
Author Affiliations:	Engineering, Division of Signals, Systems and Robotics, Zographou, 15773 Athens, Greece; <sup>2</sup> Agricultural University of Athens, Department of Agricultural Engineering, Laboratory of Agricultural Machinery and Automation, era Odos 75, Botanikos, 11855 Athens, Greece; <sup>3</sup> Department of Automatics, University of Rousse, 8, Studentska Street, 7017 Rousse, Bulgaria
ISSN:	0168-1699
Title:	In Vitro Regeneration and Genetic Transformation of Common Bean (Phaseolus vulgaris L.)-Problems and Progress.
Authors:	Veltcheva, M. <sup>1</sup> <u>mvelchevabg@yahoo.com</u> ; Svetleva, D. <sup>1</sup> ; Petkova, Sp <sup>.1</sup> ; Perl, A. <sup>2</sup>
Source:	Scientia Horticulturae; Vol. 107, 1 (2005 Dec), 2-10
Author Affiliations:	<sup>1</sup> Department of Genetics, Agricultural University, 12, Mendeleev Str., Plovdiv 4000, Bulgaria; <sup>2</sup> Department of Fruit Tree Breeding and Molecular Genetics, Agricultural Research Organization, the Volcani Center, P.O. Box 6, Bet-Dagan 50250, Israel
IS9N:	0304-4238

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Equal in European Research Area

Title:	Parasitism of Cameraria Ohridella (Lepidoptera, Gracillariidae) in Natural and Artificial Horse Chestnut Stands in the Balkans.
Authors:	Grabenweger, G. <sup>1</sup> giselher@grabenweger.at; Avtzis, N. <sup>2</sup> ; Girardoz, S. <sup>3</sup> ; Hrasovec, B. <sup>4</sup> ; Tonov, R. <sup>5</sup> ; Kenis, M. <sup>3</sup>
Source:	Agricultural & Forest Entonology; Vol. 7, 4, (2005 Nov.), 291-296, 1 chart, 1 diagram
Author Affiliations:	<sup>1</sup> Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources and Applied Life Sciences, A-1190 Vienna, Austria; <sup>2</sup> Technological Educational Institution of Kavala, Department of Forestry, Proastio, 66100 Drama, Greece; <sup>3</sup> CABI Bioscience Switzerland Centre, Rue des Grillons, 2800 Delémont, Switzerland; <sup>4</sup> Faculty of Forestry, University of Zagreb, Svetosimunska 25, 41001 Zagreb, Croatia; <sup>5</sup> Faculty of Agronomy, University of Forestry, 10, Kliment Chridski Blvd., 1756 Sofia, Bulgaria
ISSN:	1461-9555
Title:	Reviving Pre-Socialist Cooperative Traditions: The Case of Water Syndicates in Bulgaria.
Authors:	Theesfeld, Insa <sup>1</sup> ; Boevsky, Ivan <sup>2</sup> ;
Source:	Sociologia Ruralis; Vol. 45, 3 (2005 Jul.), 171-186,
Author Affiliations:	<sup>1</sup> Humboldt University of Berlin; <sup>2</sup> Institute of Agricultural Economics, Bulgaria
ISSN:	0038-0199
Title:	Growth Control of Listeria Innocua 2030c during Processing and Storage of Cold-Smoked Salmon Trout by Carnobacterium Divergens V41 Culture and Supernatant.
Authors:	Vaz-Velho, M. <sup>1,2</sup> vazvelho@estg.ipvc.pt;Todorov, S. <sup>3,4</sup> ; Ribeiro, J. <sup>1</sup> ; Gibbs, P. <sup>1,5</sup>
Source:	Food Control; Vol. 16, 6, (2005 Jul.), 540-548,
Author Affiliations:	<sup>1</sup> Escola Superior de Biotecnologia, Universidade Catolica Portuguesa, R. Dr. Antoínio Bernardino de Almeida, 4200-072 Porto, Portugal; <sup>2</sup> Escola Superior de Tecnologia e Gestao, Instituto Politécnico de Viana do Castelo, Av. Atlantico, 4900 Viana do Castelo, Portugal; <sup>3</sup> École Nationale d'Ingénieurs des Techniques des Industries Agricoles et Alimentaires (ENITIAA), Nantes, France; <sup>4</sup> Faculty of Biology, Department of Microbiology, University of Sofia, 8, Dragan Tzankov Blvd., 1423 Sofia, Bulgaria; <sup>5</sup> Leatherhead Food Research Association, Surrey, UK
IS9N:	0956-7135

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Advances in Bulgarian Science

Title:	Molecular Farming in Plants: An Approach of Agricultural Biotechnology.
Authors:	Kamenarova, Kunka <sup>1</sup> ; Abumhadi, Nabil <sup>1</sup> , Gecheff, Kostadin <sup>2</sup> ; Atanassov, Atanas <sup>1</sup>
Source:	Journal of Cell & Molecular Biology; Vol. 4, 2, 2005, 77-86,
Author Affiliations:	<sup>1</sup> AgroBioInstitute, 8, Dragan Tzankov Blvd., 1164 Sofia, Bulgaria; <sup>2</sup> Bulgarian Academy of Science, 1113 Sofia, Bulgaria
ISSN:	1303-3646
Title:	Immunomodulatory Effects of C3bgp on the Antibody Response to Hemocyanin in Outbred Rabbits and the F1 Generation of Breeding with Siblings.
Authors:	Stanilova, Spaska A. <sup>1</sup> stanilova@mf.uni-sz.bg; Miteva, Lyuba D. <sup>1</sup> ; Tanchev, Svetlin G. <sup>2</sup>
Source:	Veterinary Immunology & Immunopathology; Vol. 106, 1/2 (2005 Jun.), 15-21,
Author Affiliations:	<sup>1</sup> Department of Molecular Biology, Immunology and Genetics, Faculty of Medicine, Thracian University, 11, Armeiska Str., 6000 Stara Zagora, Bulgaria; <sup>2</sup> Department of Genetics, Breeding and Reproduction, Faculty of Agriculture, Thracian University,
	Students' Campus, 6000 Stara Zagora, Bulgaria

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Events

# SECOND NATIONAL INNOVATION FORUM TOOK PLACE IN SOFIA ON OCTOBER 25, 2005

ARC Fund organized the Second National Innovation Forum at the Sheraton Sofia Hotel Balkan on October 25th, 2005. The event was organized in cooperation with the Ministry of Economy and the World Bank Sofia Mission, with the support of DG Enterprise of the European Commission, the Austrian Embassy in Sofia, the EPIQ Electronic Assembly Company and the Center for the Study of Democracy. The Forum was held under the patronage of the President of the Republic, Mr. Georgi Parvanov.

The main objective of the Forum was to continue the dialogue and networking among the stakeholders in the country's national and regional innovation systems and serve as a platform for exchange of ideas and cooperation between the government, business community and research organizations. It additionally sought to promote the RIS (i.e. Regional Innovation Strategies) process in all planning regions of Bulgaria and the on-going RIS BRIDGE initiative for the South West region of Bulgaria. By presenting ARC Fund's annual report Innovation.bg the Forum also served as an open mechanism for benchmarking the local innovation knowledge and expertise with the European and international best practice.

The forum's agenda featured presentations by representatives of the European Commission, DG Enterprise, ARC Fund, State Agency for IT and Communications, Bulgarian Small and Medium Enterprises Promotion Agency, National Science Fund, University of National and World Economy, Bulgarian Academy of Sciences, Center for the Study of Democracy, Coventry University Enterprises Itd., UK, Cambridge University, UK, URENIO Research Unit at the Aristotle University, Greece, the Regional Governor of Stara Zagora, EPIQ Electronic Assembly Company, Ludogorie-91 Ltd. and Point L Bulgaria Ltd.

Dr. Ognyan Shentov, Chairman of ARC Fund, welcomed the participants and underlined a number of key factors for achieving qualitative economic growth amongst which he underscored the need for strong political will at the highest level, a new long-term political vision and intensive public-private partnerships.

Mr. Oscar de Bruyn Kops, Country Manager of the World Bank's Bulgaria Office, stressed upon Bulgaria's challenge to sustain high growth level and productivity despite the unfavorable demographic situation in the country. Mr. de Bruyn Kops also emphasized crucial needs for human capital improvement, labor market performance optimization and decrease in unemployment. He highlighted the support, which the World Bank provides for reforms in Bulgaria, in particular structural reforms, strengthening of institutional capacity and social sustainability.

In his keynote address President Georgi Parvanov recognized the importance of the second National Innovation Forum as an evidence for an emerging and necessary tradition. The President suggested a possible and successful approach for bridging the gap between SMEs and the pool of innovative information. This should be sought in the application of the popular formula "business trains business", as well as in the active cooperation among NGOs, professional associations, academic comunity and the partners from the EU and USA.

The forum continued with the award ceremony announcing the winners in the second national Contest for Innovative Enterprise of the year 2005. The contest was open to participants from all industrial sectors with innovative ideas, products and services. Awards were given in two main categories according to the companies' size: for small innovative enterprise (of less than 50 employees), and medium-sized and large enterprise (over 50 employees). The chairman of the jury, Prof. Marin Petrov, announced the winners in the contest and Mr. Plamen Vachkov, Chairman of the Agency for IT and Communications, handed in the awards.

The first thematic session devoted to Innovation.bg: the State and Perspectives of the National Innovation System was opened by Mr. Yannis Tsilibaris, Deputy Head of the Support for Innovation Unit at the European Commission's DG Enterprise, who gave an overview of the Commission policy on raising European competitiveness and innovation for overcoming the persistent gap of some 30 % in GDP per capita between the EU and the USA, for addressing the disparities in economic development among European regions and the variations in the regional innovation performance. Mr. Tsilibaris introduced the audience to the Competitiveness and Innovation Framework Program (CIP) and its objective to promote coordination between regions in terms of Regional Innovation Strategies. Mr. Plamen Vachkov, Chairman of the Agency for IT and Communications at the Council of Ministers listed the necessary factors for the development of innovation in the ICT sector as one of the most important sectors for the Bulgarian economy. The need for more flexible economy was stressed upon as a necessary means of deploying the potential Bulgaria has in the ICT sector - 15,000 IT specialists, which is 12% of the total number of IT specialists in Eastern Europe, 500 software companies and 110 Cisco academies. However, the investments in R&D remain highly insufficient - they are four times smaller than those in the EU countries.

Mr. Ruslan Stefanov, Project Coordinator at the Center for the Study of Democracy, presented the objective and main findings of the **Innovation.bg** Report, which is intended to facilitate the process of shaping a new long-term vision for innovation. The approach that underpinned this report is based on the consolidation of all aspects of the innovation policy in a single document. The key aspects of the EU innovation policy, as well as an overview of the new initiatives and different policies in the area of innovation were outlined by Prof. Milanka Slavova from the University of National and World Economy. She drew the audience's attention to the fact that the only strategic plan, which includes concrete measures for promotion of the innovation, is the National Plan for Regional Development. Ms. Zoya Damyanova, Program Director of ARC Fund, extended this issue by presenting the role of the RIS initiatives for the development of the regional innovation systems and successful absorption of the EU Structural Funds in the future. She announced ARC Fund's initiative of creating a National Network of RIS regions by signing a Memorandum of Cooperation, which was welcomed by the coordinators of all the other RIS projects in Bulgaria.

Within the Second Thematic Session on Regional Innovation Strategies Dr. Clive Winters, Program Director at the Coventry University Enterprises Ltd, UK, shared with the audience the experience of the West Midland Region in the RIS process and made an overview of pilot and emerging projects suggesting mechanisms for changing the innovation landscape in the region. The sharing of RIS experiences was continued by Prof. Nicos Komninos, Director of URENIO - Research Unit at the Aristotle University of Thessaloniki, Greece, who presented some successful RIS initiatives that could serve as models for wiring up a successful innovation system in Bulgaria. The experience from the implementation of the pilot RIS in the South Central Region of Bulgaria, along with some conclusions, lessons learned, and Ms. Maria Neikova, Regional Governor of the Stara Zagora Region, presented recommendations. She appealed to the coordinators of the current RIS initiatives to clearly formulate at the very beginning the expected results and measures to be undertaken so as to achieve synergy. Ms. Daniela Tchonkova, RIS BRIDGE Project Coordinator at ARC Fund, who presented the new RIS BRIDGE initiative for the South West Planning Region, concluded the session. She put strong emphasis on the specifics of the RIS BRIDGE initiative - in contrast to the standard RIS concept - which takes account of the intra-regional disparities and applies a differentiated approach tailored to the Sofia-city sub region and the rest of the SW planning region. Ms. Tchonkova explained that some foreseeable elements would also be incorporated in the RIS process and the latter should result in one Regional Innovation Strategy with two individual Action Plans for both sub regions.

The thematic session of Technology Transfer and Financing of Innovation was opened by Prof. Stefan Hadjitodorov, Secretary of the Bulgarian Acadenty of Sciences (BAS), who focused on the innovation activities of the BAS research institutes so as to build a system, which facilitates the application of science in practice. He gave a number of examples such as utilization of the innovative potential of the PhD fellowships and the effective exploitation of Internet for advertising and brokening innovation products and services. The session continued with the innovation perception from the business point of view. Mr. Nikolay Berov, Executive Director of EPIQ Electronic Assembly, Botevgrad, shared this company's experience in developing a successful innovative strategy. He stressed the importance of the human potential that needs to be well qualified and experienced as well as internally and externally motivated. The floor was taken by Ms. Kalina Miteva, Executive Director of Ludoqorie 91 Ltd., Kubrat, who presented the firm's participation in the global innovative network together with the Swedish giant IKEA. She highlighted the importance of professional cooperation and exchange of experience with foreign innovative firms. At the end of this part of the session Mr. Petar Petrov, Manager of Point L Bulgaria Ltd, Sofia - winner of the Innovative Enterprise Competition 2004 (in the category for innovative small enterprise), described the problems facing the small enterprises (micro-firms) in Bulgaria, as well as effective strategies and working mechanisms applied by Point L in overcoming significant risks and high level of uncertainty.

The concluding part of the session was dedicated to financial mechanisms for boosting innovation. Ms. Albena Vutzova, Director of the National Science Fund at the Ministry of Education and Science, pointed out that (unfortunately) most of the R&D financing comes from international programs and not from the national government or private sector. She presented the activity of the National Science Fund and its new schemes for improving the innovative performance of SMEs and scientific potential of research organizations. Ms. Vutzova recommended establishing of a monitoring system in order to trace the life cycle of innovative products as well as the consequent economic development of the company. Mr. Stanimir Barzashki, Executive Director of the Bulgarian Small and Medium Enterprises Promotion Agency, listed the instruments through which the state supports and promotes innovation-related activities of the firms with special attention to the National Innovation Fund - its objectives, types of projects supported and results of the first round of grants awarded by the Fund. Dr. Ilian Iliev from the University of Cambridge, UK, presented various forms of venture capital financing, along with the need for venture capital in support of innovation and introduction of new technologies in the context of transition economies. He underlined that the major barriers faced during the development of venture capital (VC) in CEE and Bulgaria are low level of development of the firms, lack of local VC funds, low access to networks, low managerial capacity, lack of developed networks between companies, universities and industrial partners, and low development of the clusters in which the VC could have maximum impact.

## FP6 PROJECT "RESEARCHERS IN EUROPEAN KNOWLEDGE SOCIETY" COMPLETED SUCCESSFULLY.

Conference with international participation "The Researcher – a Factor for the Development of the Knowledge Society" which took place on 28-29 November 2005 in Sofia finalized the set of activities of the "Researchers in European Knowledge Society" (REKS) project in Bulgaria http://www-it.fmi.uni-sofia.bg/REKS/index.html.

The **REKS** project addressed the **overall strategic objective** of the FP6 Human Resources and Mobility activity: *"to provide broad support for the development of abundant and dynamic world-class human resources in the European research system*". It focused on public recognition of researchers and the development of researchers' careers both at national and regional levels in Bulgaria, and paid due attention to participation of women in research.

The project formulated its **main goal** to raising the awareness of Bulgarian society on the importance of researchers for building a knowledge economy and society in Bulgaria as a part of the European ones.

The main goal of REKS was detailed into the following **project objectives**:

 facilitation of the dialogue between different stakeholders in the economy and the society on the importance of researchers and identification of the respective policy needs;

improvement of the appreciation of researchers in society, and by young people in particular;

- dissemination of information regarding practical tools supporting the researcher's career in Europe and in Bulgaria.

Beyond the stated objectives the project contributed to raising the awareness of Bulgarian society on the researchers' mobility initiatives, and facilitated the more active utilization of the available tools supporting the researcher's career in Europe.

The project implementation included the following groups of activities:

✓ Awareness-raising rally- four regional seminars, carried out in the towns of Rousse, Varna, Stara Zagora and Plovdiv dedicated to the researcher's profession.

 ✓ National conference- organized in Sofia at the end of the project. ✓ Survey on the state-of-the-art in the researcher's profession, conducted during the REKS project with results reported at the final conference.

✓ **TV broadcasting**- six integrated TV transmissions within the TV production 'Hi Tech' on the national Nova Televisia- one every month during the 'Researchers in Europe Initiative'.

✓ **Dissemination**- on-line and off-line channels for dissemination and reaching a wider audience in Bulgaria were applied.

✓ Participants in the REKS Project were as follows: Co-ordinator-Sofia University "St. Kliment Ohridski"; University of Rousse "Angel Kantchev"; Technical University of Vama; Trakia University of Stara Zagora and Paisij Hilendarski University of Plovdiv. Regional centres of the Bulgarian Network of Research Mobility Centres (RMC) also had contribution to the fulfillment of the project objectives This network was established as Bulgarian part of the ERA-MORE – The European Network of RMC (http://europa.eu.int/eracareers) in the framework of the EC Communication 'A Mobility Strategy for the European Research Area'.

The outcomes of the project can be summarized as follows:

 increased awareness of the general public and businesses on the importance of research of human resources for building the knowledge economy and society;

increased awareness on Bulgarian and Europe-wide initiatives related to researchers;

 increased awareness on career and job opportunities for young scientists in Europe;

 contribution to research of human capacity building in the country;

 minimizing weaknesses in research labour force and thus supporting the knowledge-based society in Bulgaria as a part of the European one;

 enhanced participation of Bulgarian researchers in the Marie Curie Actions, and other EU programs related to human capacity building.

The project activities had a **substantial impact** on Regional and National levels, and the project results had a clear European added value.