MINISTRY OF EDUCATION, CULTURE AND RESEARCH OF REPUBLIC OF MOLDOVA

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INSTITUTE OF MICROBIOLOGY AND BIOTECHNOLOGY
SOCIETY FOR MICROBIOLOGY OF MOLDOVA

SMM

International Scientific Conference on Microbial Biotechnology *4th edition*

CHISINAU, MOLDOVA, OCTOBER 11–12, 2018

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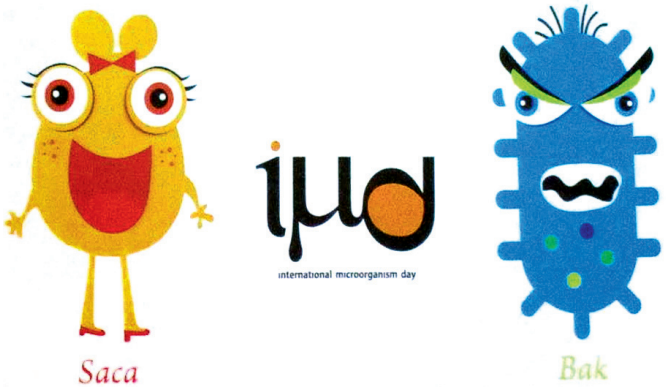
international | microorganism | day

"The role of the infinitely small in nature is infinitely great."

(Louis Pasteur)

The world is much more than you can see! There is an invisible multitude of microorganisms which are our life support. Discovering, recognizing and exploring the importance of microorganisms is a long road that is not yet appreciate by all. The aim of creating the Day of the Microorganism is to raise awareness among young people and society in general of the role of microorganisms as life supporting systems and biotools.

At the initiative of Portuguese Society of Microbiology, on **September 17, 2018,** across Europe and the world for the first time was celebrated the **International Microorganism Day**. The 17th of September was chosen to celebrate the day when the Dutch scientist Anton van Leeuwenhoek, in 1683, has sent a letter to the Royal Society of London making him the first person to observe and describe single-celled organisms, thereby launching the basis for Microbiology, one of the most important branches of Life Sciences.



PROGRAM OVERVIEW

Hotel "Vila Verde", str. Grenoble 110, mun. Chisinau, Republica Moldova

11 OCTOBER, TUESDAY	
9.00 – 10.00	*Registration*
10.00 – 10.30	*Opening (Hall B)*
10.30 – 12.00	*Plenary session (Hall B)*
12.00 – 13.00	*Networking Lunch ("Vila Verde" Hotel Restaurant) Conference photo*
13.00 – 14.30	*Plenary session (Hall B)*
14.30 – 15.30	*Poster session*
15.30 – 18.30	*Cultural program and diner*
12 OCTOBER, FRIDAY	
9.00 – 10.30	*Thematic session 4 : ● Green Biotechnology (Agricultural and Environmental Biotechnology) – Hall B* *Thematic session 1: ● Red Biotechnology (Health, Medical, Diagnostics) – Hall C* *Thematic session 2: ● Yellow Biotechnology (Food Biotechnology, Nutrition Science) – Hall C* *Thematic session 3: ● Blue Biotechnology (Aquaculture, Costal and Marine Biotechnology) – Hall C*
10.30 – 11.00	*coffee break*
11.00 – 12.30	*Thematic session 4 : ● Green Biotechnology (Agricultural and Environmental Biotechnology) – Hall B* *Thematic session 5,6: ○ ● White and Gold Biotechnology (Gene-based Bioindustries, Bioinformatics, Nanobiotechnology) – Hall C* *Thematic session 7: ● Grey Biotechnology (Classical fermentation and Bioprocess Technology) – Hall C*
12.30 – 13.30	*Closing remarks, suggestions*
14.30 – 15.30	*Poster session (Event Hall)*

PROGRAMME

11 OCTOBER, *TUESDAY* ***Venue:*** *Hotel "Vila Verde" str. Grenoble 110, mun.Chisinau, Republica Moldova*	
9.00 –10.00	***Registration***
10.00–10.30 ***Hall B***	***Opening*** **Honorific president,** acad. GHEORGHE DUCA, President of Academy of Sciences of Moldova **Representative** of the Ministry of Education, Culture and Research of the Republic of Moldova **Executive President of the Conference,** acad. VALERIU RUDIC, Director of Institute of Microbiology and Biotechnology, President of Society for Microbiology of Moldova
10.30 –12.00 ***Hall B***	***Plenary session*** **"THE PLANET OF BACTERIA": ABOUT SOME CONCEPTIONS OF THE ACADEMICIAN G.A. ZAVARZIN IN ECOLOGICAL AND ENVIRONMENTAL MICROBIOLOGY,** KOLOTILOVA NATALIA, Lomonosov Moscow State University,Russia **HYBRID STRUCTURES BASED ON MONODIMENSIONAL OXIDE NANOSTRUCTURES AND EXTREMOZYMES WITH APPLICATIONS IN AGRICULTURE,** ENACHE MADALIN, BADEA ALINA, NEAGU SIMONA, COJOC ROXANA, ANASTASESCU MIHAI, GOMOIU IOANA, ZAHARESCU MARIA, Institute of Biology Bucharest of the Romanian Academy of Sciences, Romania **NANOBIOREMEDIATION: THE EFFECTS OF NANOSCALED ZERO-VALENT IRON ON THE ACTIVITY OF THE SOIL MICROBIAL BIOMASS AND EXOGENOUS POP DEGRADING MICROORGANISMS IN SOIL POLLUTED BY POPS,** CORCIMARU S., TANASE A., COZMA V., RASTIMESINA I., POSTOLACHI O., SÎRBU T., SLANINA V., BATÎR L., CHISELITĂ O., GUTUL T.Institute of Microbiology and Biotechnology, Republic of Moldova **NANOTOXICOLOGY – EXTENSION TO AUTOTROPHIC ORGANISMS,** MOTYKA OLDŘICH,VŠB – Technical University of Ostrava, Czech Republic **NEUTRON ACTIVATION ANALYSIS AND RELATED ANALYTICAL TECHNIQUES IN BIOTECHNOLOGICAL STUDIES,** ZINICOVSCAIA INGA, Joint Institute For Nuclear Research, Dubna, Russia, HORIA HULUBEI National Institute For R&D In Physics And Nuclear Engineering, Romania
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13.00–14.30 ***Hall B***	***Plenary session*** **INCIDENCE AND ANTIBIOTIC SUSCEPTIBILITY OF BACTERIAL STRAINS ISOLATES FROM WOUND INFECTIONS,** BALAN GRETA, PUŞCAŞ NICOLAE, LOZAN-TÎRŞU CAROLINA, ZARICIUC ELENA, RUDIC VALERIU, State University of Medicine and Pharmacy „Nicolae Testemitanu", Republic of Moldova **THE ROLE OF THE MICROBIOLOGICAL METHODS IN DIAGNOSIS DELAY AND TREATMENT OUTCOME IN PATIENTS WITH DRUG-RESISTANT PULMONARY TUBERCULOSIS,** LESNIC E., MALIC A., USTIAN A., State University of Medicine and Pharmacy "NicolaeTestemitanu", Republic of Moldova **FOODBORNE MICROBES & LIGHT DURING STORAGE,** YABLONSKA OKSANA, SAVCHUK OLEXANDR, MEKH NATALIA, KASPER VYACHESLAV, National University of Life and Environmental Sciences of Ukraine **PHYSICOCHEMICAL, ANTIOXIDANT AND SENSORY CHARACTERISTICS OF WINE FROM *FLACOURTIA MONTANA* FRUITS,** DEVARAJAN THANGADURAI, MUNDARAGI ABHISHEK CHANNAYYA, SANGEETHA JEYABALAN, Karnatak University, India **MANIFESTATION OF EPIZOOTIC EFFECTS OF ENTOMOPATHOGENIC INFECTIONS,** LEONID VOLOSCIUC, Institute of Genetics, Physiology and Plant Protection, Republic of Moldova
14.30–15.30 ***Hall B***	***Poster session***
15.30 – 18.30	***Cultural program and diner:*** Visit to the one of the famous Moldovan wine cellar.

12 OCTOBER, *FRIDAY* ***Venue:*** *Hotel "Vila Verde" str. Grenoble 110, mun.Chisinau, Republica Moldova*	
9.00 – 10.30 ***Hall B***	*Thematic session 4:* • **Green Biotechnology (Agricultural and Environmental Biotechnology)** **EFFICIENCY OF NEW SORBENTS BASED ON CHITOSAN FOR THE REMOVAL OF HEAVY METALS FROM RESIDUAL WATERS,** Humelnicu D., Dragan E., Dinu M., Zinicovscaia I., Humelnicu I., „Al. I. Cuza" University of Iasi, Romania **SCREENING OF BACTERIA PRODUCING EXTRACELLULAR HYDROLYTIC ENZYMES FROM MANGROVE SEDIMENTS,** Sangeetha Jeyabalan, Anjana R., Steffi Simmi Maxim, Devarajan Thangadurai, Central University of Kerala, India. **EFFECT OF PLANT GROWTH PROMOTING BACTERIA (PGPB) ON COPPER TOXICITY REDUCTION IN GRAPE SEEDLINGS,** Veliksar S., Lemanova N., Zacchini M., Pietrini F., Gladei M., Institute of Genetics, Physiology and Plant Protection, Republic of Moldova, Institute of Agroenvironmental and Forest Biology, National Research Council, Italy **MOLECULAR DETECTION OF *CAMPYLOBACTER, BRUCELLA* AND *LEPTOSPIRA* SPP. IN BREEDING BULLS OF PUNJAB, PAKISTAN,** Saher Islam, Wasim Shehzad, Yung-Fu Chang, University of Veterinary and Animal Sciences, Lahore, Pakistan; College of Veterinary Medicine, Cornell University, Ithaca, New York, USA **STUDY OF CHEMISTRY OF CR(VI)/CR(III) BIOSORPTION FROM BATCH SOLUTIONS AND ELECTROPLATING INDUSTRIAL EFFLUENT USING CYANOBACTERIUM *SPIRULINA PLATENSIS*,** Yushin N., Zinicovscaia I., Cepoi L., Chiriac T., Mitina T., Dubna University, Russia. **AFFINITY OF *IRIS* × *HOLLANDICA* TO ARBUSCULAR MYCORRHIZAE DURING ESTABLISHMENT IN THE FIELD**, Crişan I., Vidican R., Stoian V. , Şandor M., University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania **MYCORRHIZAL DYNAMICS IN ROOT OF URBAN *TRIFOLIUM* GENUS**, Stoian V., Vidican R., Şandor M., Crişan I., Blanariu M., Pleşa A. University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania **ENTOMOPATGENIC BIOPESTICIDE IN PROTECTING *LEPIDOPTERA* DIVERSITY IN ORGANIC FARMING**, Stingaci A., Institute of Genetics, Physiology and Plant Protection, Republic of Moldova
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• Yellow Biotechnology (Food Biotechnology, Nutrition Science)

THE INFLUENCE OF BioR AND BUTOFAN REMEDIES ON THE HEALTH AND PRODUCTIVITY OF ADULT QUAILS UNDER RECONDITIONING, Macari V., Ruduc V., Pavlicenco N., Rotaru A., Putin V., Alzinati M., Enciu V. , State Agrarian University of Moldova, Institute of Microbiology and Biotechnology, Republic of Moldova

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○ • White and Gold Biotechnology (Gene-based Bioindustries, Bioinformatics, Nanobiotechnology)

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ISOLATION AND OBTAIN OF PURE YEAST STRAINS FROM THE "TRIFESHTI" VINEYARD FOR PRODUCTION OF DRY WHITE AND RED WINES, Soldatenco O., Public Institution Scientific And Practical Institute Of Horticulture And Food

Conference Proceedings

In memoriam

PETRU GALEȚCHI (1938-2004)

On September 6, 2018, we marked the 80th birthday of Professor Petru Galețchi - a true pylon of the university, a personality who leaved remarkable traces in science, in organizational activity, but especially in medical pedagogy.

He was born in Dondușeni village, in a family of farmers, were he was educated to work, to be honest, to defend his dignity, since he was a young child. Throughout his live, these qualities, inherited and tormented in time, have been honored with holiness.

Petru Galețchi successfully graduated from Bălți School of midwifery in 1956 and graduated with a Diploma with mention from the State Institute of Medicine in 1963.

Since 1966 he has been studying the infectious process and conducting a series of experiments in the laboratory of Infectious Pathology and Experimental Therapy at the Institute of Epidemiology and Microbiology "N. Gamaleia "in Moscow.

In 1970, he defended the thesis of doctor of medical sciences in the issue related to the particularities of the evolution of the infectious process caused by antibiotic-resistant Staphylococci under the action of penicillin therapy. One of the conclusions of the paper is written by the author as follows: "Currently, the epidemiology of staphylococcal infections denotes a widespread presence of these microbes among sick people, the bmedical staff and the population, which is largely determined by the persistent circulation of antibiotic-resistant staphylococci." This problem remains current.

Since 1975 Mr. Petru Galețchi worked as associate professor at the department of microbiology, virology and immunology, and since 1989 – as head of the chair. In 1989-1995 he served as Dean of the General Medicine Faculty, and in 1995 he was appointed Vice-Rector and First Vice-Rector, a position he fulfills with all responsibility until August 31, 2004.

Vice-rector Petru Galețchi contributed to optimization of the didactic process by implementing modern training and evaluation technologies, introducing new forms of organization of university education, including the application of interdisciplinary modules and transferable academic credits, the development of new educational standards, the completion of study plans in accordance with the requirements of the World Health Organization and the European area.

Professor Petru Galețchi created a team of experienced staff (deans, pro-deans, etc.) who, developed the concept of continuing training of graduate and postgraduate doctors and pharmacists from all country.He has performed a fruitful activity in the evaluation and accreditation process of the State University of Medicine and Pharmacy "Nicolae Testemitanu".

Prof. Petru Galețchi is well known and appreciated as a member of the University Senate, the Scientific Council and Senate Bureau, the Board of Directors, the Specialized Scientific Council, the President of the Methodical Academic Commission and many others.

In memoriam

ŞTEFAN PLUGARU: 1938-2010

It is 8 years since he passed away… The scientist, Ştefan Plugaru, the head of the Department of Microbiology, Virology and Immunology, would be celebrated his 80th birthday.

Stefan Plugaru was born on July 26, 1938, in the village of Bardar, Lăpuşna County (now Ialoveni district). He graduated from the Pedagogical College of Călăraşi in 1957 and from the State Medical Institute in 1966. His professional career has evolved through the characteristic steps of the university professor:

• From 1966 to 1993 he worked as assistant, then senior lecturer, associate professor at the Department of Microbiology, Virology and Immunology of the "Nicolae Testemiţanu" State University of Medicine and Pharmacy.

• In 1973 he successfully defended the doctoral thesis in medicine: "Development of new and rapid methods in the diagnosis of intestinal infections".

• From 1972 to 1975 he worked as dean of the Faculty of Medicine, during which he gained new experiences, demonstrating organizational and pedagogical abilities.

• Between 1975 and 1988 he continued his activity as Dean of the Faculty of Public Health, contributing of improving and modernizing the study process, maintaining discipline in the training of doctors.

• During 1993 - 2002 he worked as a director of the Republican College of Medicine and Pharmacy, today the National College of Medicine.

• Since 2004 he became head of the Department of Microbiology, Virology and Immunology.

During the years he worked as a Principal Microbiologist of the Ministry of Health, member of the SUMF Senate, member of the Council of General Medicine, member of the Scientific Council, member of the editorial board of the journal "Public Health", member of the Commission for Attestation of Microbiologists of the Ministry of Health of the Republic of Moldova, Member of the Commission on Hygiene and Public Health, member of the Scientific Council of SUMF "Nicolae Testemitanu".

It has to be mentioned the active participation of prof. Plugaru in the elaboration of manuals, compendia and practical guides of medical microbiology, widely used by General Medicine and Public Health students. During these years Professor Ştefan Plugaru published over 120 scientific papers, including three monographs (signed with Prof. V. Nikitin and other contributors) and a book, written in collaboration with prof. P. Galeţchi and prof. D. Buiuc, Iaşi (România).

For special merits in his multilateral work of scientist and teacher, in 1990, he was awarded the honorary title of Om Emeritus. For professional activity over several decades, in 2005, he was decorated with the "Gloria Muncii" Order.

By his special attitude towards the professional and scientific obligations, the erudition and pedagogical tact, the well organized way of life and excellent personal qualities the scientist Ştefan Plugaru earned the well-deserved respect and love of his colleagues, disciples and students.

The staff of the Department of
microbiology and immunology,
State University of Medicine and
Pharmacy "Nicolae Testemitanu"

Plenary Session

"THE PLANET OF BACTERIA": ABOUT SOME CONCEPTIONS OF THE ACADEMICIAN G.A. ZAVARZIN IN ECOLOGICAL AND ENVIRONMENTAL MICROBIOLOGY

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Microorganisms play today a very important role in different fields of biotechnology, what is in particular illustrated by this conference. Our comprehension of their importance is reflected in the celebration of "the International Microorganism Day", the selection of "the Microbe of the Year" etc.

But certainly the role of microbes in the biosphere is much greater then that from our anthropocentric point of view. Some ideas of the outstanding Russian microbiologist G.A. Zavarzin (1933–2011) must be mentioned. Developing the conceptions of the other great microbiologist, S.N. Winogradsky (1856-1953) who had shown the role of bacteria in the biosphere as catalysts of chemical reactions in main biogeochemical cycles, G.A. Zavarzin wrote about the central role of bacteria (prokaryotes) in the development of the biosphere and as a consequence about the central place of microbiology in biology and even in natural science. These are bacteria (prokaryotes) that had created the biosphere (which was in the Precambrian the "bacteriosphere") and continue today to regulate the main biogeochemical cycles, being the basis of the biosphere while the eukaryotes are the superstructure. This idea is reflected also in the Zavarzin's conception of "the additional evolution".

Many ideas of G.A. Zavarzin in global microbiology can be used successfully in biotechnology, especially"green".

G.A. Zavarzin leaved a great scientific heritage. Today many of his biologicaland philosophical works are of current interest. That's why the aim of this report is also to present some of his books and other publications that were reedited during last years.

HYBRID STRUCTURES BASED ON MONODIMENSIONAL OXIDE NANOSTRUCTURES AND EXTREMOZYMES WITH APPLICATIONS IN AGRICULTURE

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Introduction

Soil is an important part of a terrestrial ecosystem and provides the fundamental support for all forms of terrestrial life. In this way, adequate soil protection programs are very necessary to avoid erosion, infertility, contamination of subterranean waters and, last but not least, loss of biodiversity. Soil quality is correlated with its biological properties that are affected by any changes in the environment. Also, soil microbiology is sensitive and changes rapidly in response to any changes in the environment. Soil microbiota profiles and enzymatic systems are closely correlated and represent an important indicator of soil health and quality (Pajares et al., 2011; Joshi et al., 2018). Soil enzymes, which are mainly synthesized by soil microorganisms, play a crucial role in the nutrient circuit and reflect microbiological soil activity and fertility (Bentez et al., 2000). Soil microorganisms mediate biochemical processes together with plants (their roots) and animals present in the soil. Biochemical processes are catalyzed by a series of enzymes found in the soil, including: glucosidases, xylozidases, amylase, dehydrogenase, chitinase, urease, proteases, phenol oxidases, aminopeptidases, phosphatases.

There it is of high scientific and economical interest to modulate and bring together one of the most efficient and fragile natural mechanism (the enzymatic one) with a synthetic well defined and very promising oxidic (tubular/1D) nanostructure. Many attempts are done in order to immobilize and encapsulate biological active species, especially enzymes, in appropriate inorganic matrices, usually mesoporous silica with pore diameters covering a range of 15-300 Å, this range being compatible with molecular diameters of enzymes (Takahashi et al., 2000). We have obtained highly reproducible SiO_2 nanotubes by sol gel method using the tartaric acid as organic template in the reaction mixture, in mild conditions. The hollow tubes have an external diameter of about 350 nm, an internal one of about 200 nm, open ends, a high surface area ($300m^2/g$) and lenghts around micrometers (Anastasescu et al., 2010). The tubular SiO_2 matrix is appropriate for doping and catalytic reactions (Anastasescu et al., 2012), has an intrinsic chemical reactivity (because of its large band gap, up to now was considered just a good support material) and is also a light sensitive material, a slight photocatalytic activity being registered even for pure silica (Anastasescu et al., 2009). The obtained TiNTs nanotubes had also reproducible tubular morphology but much smaller diameters, and a high surface area (Preda et al., 2013). We considered the morphological characteristics of SiO_2 and TiNTs appropriate for bioremediation processes conducted in the presence of halotolerant bacteria (Merciu et al., 2009). Based on these results, we managed to go further looking for a protease imobilization on SiO_2 and TiO_2 nanotubes (Merciu et al., 2014).

Results

The obtained results demonstrate a more pronounced alkaline phosphatase activity in the soil samples coupled to the hybrid system formed on the basis of the immobilization of the proteases on the inorganic support, compared to the soil samples coupled to the immobilized amylases and lipases on the oxide substrate. The same manifestation of the activity of alkaline phosphatase was also observed with respect to the anthropic soil sample, but also to the soil treated with the inorganic structure materials. The results were comparable for both inorganic matrices tested, the highest value being obtained for variant which has titanium dioxide as an inorganic substrate. The urease activity of the hybrid system based on the immobilization of the enzymes of interest on the inorganic support (silica) was noted by values superior to the anthropogenic soil sample. Dehydrogenase recorded activity in all tested variants. Compared to the activity value in the anthropogenic soil sample, a significant increase in activity values in soil treated only with nanomaterials variants was observed.

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NANOBIOREMEDIATION: THE EFFECTS OF NANOSCALED ZERO-VALENT IRON ON THE ACTIVITY OF THE SOIL MICROBIAL BIOMASS AND EXOGENOUS POP DEGRADING MICROORGANISMS IN SOIL POLLUTED BY POPS

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Nanoscaled zero-valent iron (NZVI) is widely studied and used as means of environmental remediation, including in cases of soil pollution by persistent organic pollutants (POPs). NZVI is also recommended for nanobioremediation, i.e. for improving the microbial activity in the polluted soils, and, consequently, stimulating soil bioremediation processes. The purpose of this work was to estimate the impact of NZVI on the soil microbial biomass (SMB) and the activity of exogenous POP degrading microorganisms in a soil characterized by long-term pollution by POPs. The studied NZVI (1.5-2.5 nm) was stabilized by poly-N-vinylpyrrolidone. It was introduced into soil in the form of crystal powder mixed with talc. The polluted soil (sampled from a former pesticide deposit site) contained 2 mg/kg of DDTs and 30 mg/kg of trifluralin.

The introduction of NZVI (100 mg/kg) into the polluted soil didn't cause substantial increases of the SMB within the first 30 days of incubation (the subsamples of treated and untreated soil were kept in the dark, moistened at 40% of water holding capacity, aerated, and at the constant temperature of 25° C). The only statistically significant effect was observed by day 16 when the SMB (in the treated soil subsample) was 8.6% higher than in the control (with talc). The greatest effects of NZVI on the SMB (up till +27.3% as compared to the control) were observed in cases when the soil was additionally treated by two consortia of exogenous microorganisms (fig. 1) selected for their ability to actively grow in media containing NZVI and trifluralin (the latter as the only source of carbon). The SMB in the soil subsamples with the consortia but without NZVI was smaller than in the parallel cases with NZVI. It was also observed that within the first 4 days of the incubation NZVI was able to substantially (by 16-18 times) increase the number of CFU of at least one of the microbial strains introduces into the polluted soil with the consortia (fig. 2).

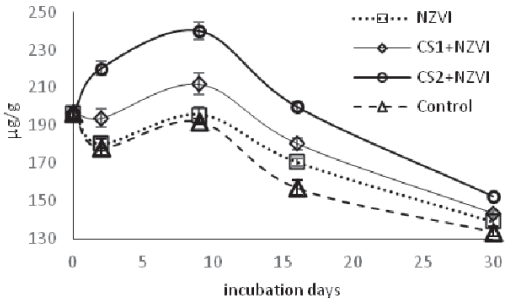

Fig. 1. The dynamics of the soil microbial biomass carbon in different subsamples of the polluted soil. Control – the subsample treated with talc, NZVI – the treatments with nanoscaled zero-valent iron, CS1 and CS2 – the treatments with 2 exogenous consortia of microorganisms.

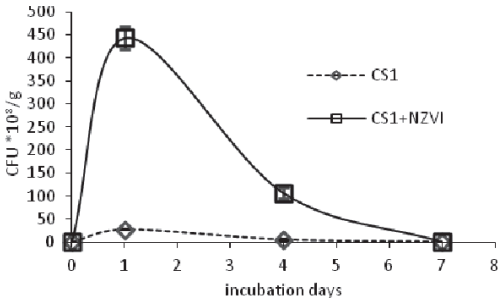

Fig. 2. The dynamics of the exogenous *Bacillus sp*. n. 2 CFU numbers obtained from different subsamples of the polluted soil, treated with the consortia of microorganisms (CS1) in the presence or absence of nanoscaled zero-valent iron (NZVI).

Conclusions: (1) NZVI can have substantial positive effects on the SMB and exogenous microorganisms introduced into soil for bioremediation purposes; (2) the effects from a single NZVI treatment decrease with time, implying need for repeated treatments; (3) Within the studied conditions NZVI treatments did not cause any negative influence on the SMB and, thus, did not imply any environmental risk; (4) NZVI can be successfully used for nanobioremediation.

NANOTOXICOLOGY – EXTENSION TO AUTOTROPHIC ORGANISMS

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Although atmosphere has a natural background level of nanoparticles, their local concentrations and purity are rather insignificant in comparison with those nanomaterials originating from the human activities The nanomaterials can be manufactured on purpose (engineered nanomaterials), but substantial portion of anthropogenic airborne nanoparticles are, however, unwanted or incidental originated from by-products of various types of combustion, food cooking, and chemical manufacturing; welding or refining and smelting. Especially nanoparticles originating in combustion are becoming of higher concern, especially due to the ever-growing production of waste that is being taken care of in incineration plants. One of the most commonly used engineered nanomaterials are metal oxides, while nanosized zinc oxide (nano-ZnO) and nano titanium dioxide (nano-TiO_2) are the most extensively used in a number of fields and applications.

It is, thus, conceivable that their unwanted presence in the environment will continue to grow. As well increasing presence of a compound in the environment, in turn, leads to the heightened need for the assessment of its impact on living systems. While attention was originally paid mostly to the research of potential toxic effects nanomaterials may have on bacterial or human (and hence animal) cells and bodies, number of studies on how the nanomaterial exposure may affect photosynthetic, autotrophic organisms has significantly grown only recently.

In the studies performed at our department, experiments on model photosynthetic organisms (bryophytes) and crops (cauliflower) exposure to nanoparticles (nano ZnO, nano TiO_2) were carried out in order to assess the uptake, distribution and effect on plant metabolism. Nanoparticles were found to penetrate the cells of bryophytes and accumulate. Nanoparticle exposure had a paradoxical positive effect on cellular membranes, yet, judging from the concentration of L-ascorbic acid determined, still caused significant oxidative stress in plant models. Nanoparticle exposure both via roots and leaves was found to lead to decreased concentrations of chlorophyll in cauliflower experiments.

NEUTRON ACTIVATION ANALYSIS AND RELATED ANALYTICAL TECHNIQUES IN BIOTECHNOLOGICAL STUDIES

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Experience in applying neutron activation analysis at the IBR-2 pulsed fast reactor in Dubna to biotechnological studies is presented. The principle of the method, the principal units of the pneumatic system REGATA are described as well as the main present-day parameters of the irradiation channels. Examples are given from two challenging areas: wastewater treatment and nanoparticles production, where neutron activation analysis together with other analytical techniques was used.

Biotechnology of wastewater treatments using microorganisms for removal of heavy metals offers an alternative to conventional techniques due to its low cost and high efficiency. The results on the use of cyanobacteria *Spirulina platensis* in the removal of wide range of chemical elements (chromium, zinc, nickel, ect.) from batch solutions and industrial effluents are reported. Spirulina showed to be an efficient biosorbent for metal removal with the efficiency ranging from 60 to 90%.

Two complementary analytical techniques: atomic absorption spectrometry and neutron activation analysis (ENAA) – were used in these studies. Fourier Transform Infrared technique allowed revealing changes in the chemical structure of cyanobacterial biomass after interaction with metal solutions.

Currently, great attention is paid to the biological synthesis of nanoparticles. *Spirulina platensis* was also applied for the synthesis of gold and silver nanoparticles. The complex of optical and analytical methods was applied for investigation of experimental samples after exposure to chloroaurate ($HAuCl_4$) and silver nitrate ($AgNO_3$) solution at different doses and for different time intervals. To characterize formed nanoparticles, UV-vis Spectrometry, X-ray diffraction, Transmission Electron Microscopy, Scanning Electron Microscopy, and Energy-dispersive analysis of X-rays and neutron activation analysis were used. It was shown that spirulina is capable of producing extra- and intracellular crystalline gold and silver nanoparticles of spherical shape when exposed to suitable compounds. The particle size distribution shows that the sizes of nanoparticles are in the range of 5 nm to 80 nm. The biomass obtained may be used for industrial as well as medical and pharmaceutical purposes.

BACTERIAL PROFILE AND ANTIBIOTIC RESISTANCE IN PATIENTS WITH TROPHIC ULCERS

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Trophic ulcer is a very common problem, occurring mainly in older people. Particularly at risk of chronic ulcers are overweight people with heart failure or diabete suffering persons. The microflora of trophic ulcers is usually polymicrobial. *Staphylococcus aureus* and coagulase-negative staphylococci have been the predominant organisms isolated from both prospective, purpose-collected samples and retrospective analysis of clinical investigations. *Pseudomonas aeruginosa* is another frequently identified organism and has been found in 7–33% of ulcers. A number of other aerobic species have also been reported, including *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella* species, *Streptococcus* species, *Enterococcus* species and *Proteus* species.

Material and methods: Totally, 108 isolates were collected from the 57 patients in different wards of the Republican Clinic Hospital. All the specimens were sampled to the microbiology laboratory within 48 h after hospital admission. No antimicrobial agent or antiseptic was introduced into the ulcer before specimen collection. The specimens were placed into sterile transport containers and sent to the microbiology laboratory for aerobic culturing within 30 minutes. Anaerobic culturing was not performed in this study. Antimicrobial susceptibility was determined using the disk diffusion method in accordance with the performance standards, recommended by the EUCAST (European Committee on Antimicrobial Susceptibility Testing).

Results: A total of 108 isolates were detected from the 57 specimens, including 98 (90,7%) bacteria and 10 (9,3%) funguses. In the bacterial infection, the proportion of Gram-negative bacteria (55,6%, 60/108) was higher than Gram-positive bacteria (44,4%, 48/108). Enterobacteriaceae was the main Gram-negative bacteria (71,7%, 43/60), mainly including *Escherichia coli*, *Enterobacter cloacae*, and *Klebsiella pneumonia. Proteus* (18,3%, 11/60) and *Pseudomonas* (13,3%, 8/60) followed. *Staphylococcus* spp. (66,7%, 32/48) is the predominant pathogen of Gram-positive bacteria, main of which was *Staphylococcus aureus* (45,8%, 22/48), followed by *Enterococcus* (18,7%, 19/48). *Candida* was the main pathogen in fungal infection, accounted for 90,0% (9/10). MDR (multiple-drug resistance) isolates were broadly distributed in the 108 bacteria isolated from trophic ulcers (42,6%, 46/108). High resistance rates to the common antibiotics were detected in Enterobacteriaceae. Almost all the isolates were resistant to the ampicillin (90,0%, 54/60), followed by the first/second generation cephalosporin, including cefazolin (78,3%, 47/60) and cefuroxime (65,0%, 39/60). Low resistance rates were detected to carbapenem (8,3%, 5/60), cefoperazone-sulbactam (13,3%, 8/60), the fourth generation cephalosporin (15,0%, 9/60), and tobramycin (16,7%, 10/60). As the representative of Gram-positive cocci, *Staphylococcus aureus* showed a high resistance rate to common antibiotics. High resistance rate to oxicillin was detected (91,7%, 44/48), followed by the tetracycline 60,4%, 29/48). The low level of resistance shown by the *P. aeruginosa* isolates to ceftazidime (a third generation cephalosporin), ciprofloxacin and gentamicin indicates that these three drugs are still effective in our country compared to other studies which showed, for example, that *P. aeruginosa* is highly resistant to gentamicin.

Conclusions: Different bacterial profiles and antibiotic sensitivity were found in trophic ulcers. Clinician should try to stay updated in antibiotic resistance pattern of common pathogens in their area, especially when practice on the empirical antibiotic use.

THE ROLE OF THE MICROBIOLOGICAL METHODS IN DIAGNOSIS DELAY AND TREATMENT OUTCOME IN PATIENTS WITH DRUG-RESISTANT PULMONARY TUBERCULOSIS

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Tuberculosis (TB) is one of the most important challenges for the health care system of any state. In 2016, 9 million new cases were registered globally, the Republic of Moldova ranking among 30 countries with the biggest burden of multidrug-resistance tuberculosis (MDR-TB). One of the most relevant control actions represents the early case detection, especially of MDR-TB. The precocious treatment is considered the most efficient tool for interrupting the epidemiological chain of disease transmission. The cultural methods remain the golden standard for TB diagnosis, despite low sensibility and long duration of cultivation. Conventional microscopy for identification of acid-fast-bacilli is the first step in the microbiological investigation. Its low sensibility endangers the epidemiological situation.

In 2011 the WHO established conditional recommendations to use Xpert MTB/RIF assay in testing adults, children and persons with HIV suspected for TB, or for testing the non-respiratory specimens targeting the diagnosis of extrapulmonary TB. It is an in-vitro diagnostic device, owned by Cepheid Company and is a semi-nested, quantitative, real-time polymerase chain reaction for the DNA detection of all *Mycobacterium tuberculosis (MBT)* complex species and rifampicin resistance mutations of the *rpoB* gene. The system automates the sample processing, nucleic amplification and detection of the target sequences of *rpoB* gene. Any biological specimen can be processed considering that it requires the minimum of 2 ml of sample volume. The high sensitivity (97,3%) of the Xpert MTB/RIF among culture positive specimens and 99.5% in smear positive patients contributes to earlier detection and precocious treatment according to the rifampicin resistant results.

The aim of the research was to establish the impact of the microbiological methods in diagnosis delay and treatment outcome in patients with MDR-TB.

Material and methods: It was performed a selective, descriptive and retrospective study conducted according to a linear model, carried out on 226 new cases of pulmonary MDR-TB investigated according to the National Clinical Protocol. Were used laboratory examinations: general blood and urine analysis, chest X-ray, sputum microscopy for acid fast bacilli, conventional microbiological investigations (smear microscopy, Lowenstein – Jensen culture, BACTEC assay) and innovative platform of the Xpert MTB/RIF test. Patients were distributed in a study group (1st group) 85 cases with MDR-TB detected through Xpert MTB/RIF test and the control group (2nd group) 141 cases treated for MDR-TB, according to the results of the drug susceptibility test on conventional microbiological methods (Lowenstein – Jensen culture either BACTEC assay).

Results and discussions. Assessing the groups according to the sex distribution was established the predominance of men in all groups. So, in the 1st group were 61 (71.7%) and women 24 (28.3%) with a male/female ratio = 2.5/1 and in the 2nd group 106 (75.1%) were men and

35 (24.8%), ratio=3/1. Detection particularities established the predominance of the passive case-finding 68 (80.1%) in the 1st group and 106 (75.2%) patients in the 2nd group. Assessing the diagnosis delay, it was identified that each fourth patient in both groups were diagnosed in more than three months after the disease onset. The early diagnosis till 30 days after the disease onset was established in each fifth patient in the 1st group 7 (8.4%). Pulmonary infiltrative TB was diagnosed in the most of the patients in both groups: 79 (93.1%) in the 1st group and 132 (93.6%) in the 2nd group. Severe forms were in a limited number 6 (7.1%) in the 1st group and 7 (4.9%) in the 2nd group. Treatment success was in a higher rate in the 1st group 59 (69.4%) vs. the 2nd group 84 (59.6%). Were loss to follow-up more frequently patients from the 2nd group 21 (14.9%) vs. 9 (10,6%). Died in a similar rate 22 (15.6%) in the 2nd group compared and 12 (14.1%) in the 1st group.

Conlcusions: Xpert MTB/Rif assay diminish the rate of late detected forms of pulmonary TB, adapts the treatment according to the resistance to Rifampicin and improves disease outcome.

Key words: tuberculosis, microbiology, Xpert MTB/RIF.

FOODBORNE MICROBES & LIGHT DURING STORAGE

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The migration of humans, animals and birds has led to the emergence of a large number of microorganisms which are not characteristic for a particular human habitat. The lack of basic hygiene conditions leads to food contamination by microorganisms from the intestines of animals / birds and trough contaminated hands of people touching these products during the slaughtering animals, packing their carcasses, transporting meat and organs, manufacturing, storing and selling products. At the same time, microorganisms have acquired genetic resistance to the high and low temperatures, which must be considered during their microbiological diagnosis in the laboratory. The use of light with different wavelengths, for illumination and signals, is more frequent during food storage.

Purpose: To study the effect of light of different lengths on the microflora of food products during their storage.

Materials: 1. Meat purchased in supermarkets; 2. Microorganisms derived from this meat; 3. Light of different length: ultraviolet - 405 nm, green light - 570 nm, red light - 750 nm and red 700-750 nm. Methods of research: microbiological, analytical, and statistical.

Research results. In 20% of pig meat in vacuum packages we have identified *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium novi*, *E. coli*; in 26% of chicken meat we found *E.coli* , *Salmonella enteritidis*, *Enterococcus,* sulfite-reducing *clostridia* (SRC), anaerobic microorganisms NMAFAnM, lactic acid bacteria, yeast and mold . The remaining part of the meat samples was sterile.

We inoculated the microbial cultures on meat-peptone broth (MPB) and meat-peptone agar (MPA). Experimental microbial cultures were illuminated during 10 seconds, 20 seconds, 30 seconds, 1 min and 2 minutes. The cultivation of samples was performed in thermostat at the temperature of 37^0C for 24-72 hours, followed by the storage in refrigerator at the temperature of $+4^0$C for 24-72 hours.

Our experiments showed different sensitivity of microorganisms to illumination. Green light illumination during 2 min exhibit a stimulating effect on the growth of *E. coli* and *Pseudomonas aeruginosa* in MPA and MPB, but *Pseudomonas aeruginosa* grows better in MPB.

The colonies of *Staphylococcus aureus* on MPA exposed to ultraviolet light have changed the saturation of the pigment and have lost the ability to form colonies. Staphylococcal control colonies retained the pigment saturation. The microscopy of staphylococci has shown aglomerate clusters of cells in experimental cultures. At the same time, we observed dependence between the growth of enterococci cultures and the period of exposure.

UV light, at 2 min exposure, exerted a slightly inhibitory action on the culture of *Clostridium novyi;* and the red laser showed a bactericidal effect, reducing the amount of *Clostridium novyi*, but caused the adhesion of cells and the formation of spores. The red laser, at 30 sec exposure, caused the rounding up of *Salmonella enteritidis* cells.

Conclusions. The analysis of the obtained results shows that the microflora of the samples under investigation does not meet the veterinary and sanitary norms. We found that chicken meat in Ukrainian markets may be a source of risk of food-borne toxicoinfections for the consumer. The effect of storage temperature and lighting is of great importance for most products that have a short shelf life and are used by children or the elderly. Experiments on the effects of lighting on microorganisms continue.

PHYSICOCHEMICAL, ANTIOXIDANT AND SENSORY CHARACTERISTICS OF WINE FROM FLACOURTIA MONTANA FRUITS

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The research rationale of the present study was to produce fruit wine from a wild fruit *Flacourtia montana* J. Graham.

The various physicochemical attributes including total phenolic content and total flavonoid content were analyzed. Further, the prepared wine was evaluated for the antioxidant potential using four different assays, *viz*., 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), reducing power assay and total antioxidant activity. Finally, the wine was subjected for the sensory evaluation.

Experimental results revealed that wine had an alcohol content of 7.20%, total phenolic content of 0.776±0.032 mg GAE/ml and total flavonoids of 0.121±0.012 mg QE/ml. High performance liquid chromatography analysis revealed the presence of four major phenolic acids, *viz*., gallic acid (0.009±0.0005 mg/ml), chlorogenic acid (0.623±0.091 mg/ml), catechin (0.063±0.011 μg/ml) and epicatechin (0.060±0.009 mg/ml). *In vitro* antioxidant analysis of wine was able to successfully scavenge the free radicals in a dose dependent manner. Sensory scores indicated wine to be good in overall acceptability.

Thus, present study highlighted the therapeutic nature of wine prepared from this underutilized fruit which could provide possibilities for enhancing socio-economic benefits among rural communities.

Keywords: *Flacourtia montana*, Underutilized fruit, Fruit wine, Polyphenols, Antioxidant activity, Physicochemical properties, Sensory evaluation.

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MANIFESTATION OF EPIZOOTIC EFFECTS OF ENTOMOPATHOGENIC INFECTIONS

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Microbial pathogens, especially baculoviruses, have an important role in population dynamics of their hosts. Nucleopolyhedrovirus (NPV) and granulovirus (GV), identified and researched by us (Ciuhrii M., Volosciuc L., 1990; Volosciuc L., 2000, 2016), as well as their hosts, phyllophagous insects experiencing population explosions, are studied in terms of the baculovirus-insect interaction. Increased selectivity, high efficacy, epizootic nature, harmlessness to humans, plants, and animals have determined both the important role of baculoviruses in regulating the density of natural insect pest populations and the possibility of widespread production and application of viral insecticides. Entomopathogenic viruses - as natural regulators of pest insect density - are increasingly being researched and applied to the biological protection of plants. Preparations based on entomopathogenic viruses, unlike chemical insecticides, have a number of advantages. Viral insecticides provide high biological efficiency; has the effect of post-action at all stages of development and controls population density over several generations; is characterized by the selectivity of the action without affecting the development of entomophagous and pollinating insects.

The application of modern research methods (Electronic Microscopy, Restriction Analysis, Polymerase chain reaction) allowed the determination of the biological features of baculoviruses in the main insect pests. The production of baculoviral preparation technologies included research to obtain technological processes of mass-growth of host insects and to determine the relationship between them and the pathogen.

For the purpose of developing biological means of controlling insects, the main indications determining the biological efficacy of baculoviruses have been established, among which their epizootic nature is particularly important. The main ways of transmitting baculoviruses in complex ecosystems and agrocenoses were determined and the epizootic nature of baculoviruses of colonial insects (*Hyphantria cunea, Lymantria dispar*) and noctuides (*Helicoverpa armigera, Mamestra brassicae*) was determined, which was allowed development of application technologies and determined the place of baculoviral preparations in the protection of vegetable, fruit and forest crops. It has been demonstrated the possibility and elaboration of original ways of applying entomophages as vectors of baculoviral infection, which greatly increases the yield of baculoviral insecticides and entomophages in protecting crops. The possibility of applying epizootic mechanisms in the control of *H.armigera* and *Carpocapsa pomonella* was determined.

Forest ecosystems are one of the most convenient models to study natural processes of interaction between biocenotic components and as results, population explosions of forest insects, can degrade the stands over considerable areas. The mechanisms underlying persistent viral infection in insect populations and initiation of massive baculovirus diseases during natural epizootics are the subjects of a long discussion. We have demonstrated that the process of baculovirus epizootic depends on a set of various factors. The efficiency of the host-parasite interaction depends on the virus biological activity, multiplicity of infection, biological properties and physiological state of the insect, properties of the food plants, and environmental

factors. Based on the data obtained by electron microscopy and methods of molecular biology, it studied the mechanisms underlying the development of epizootic, a relatively underexplored natural phenomenon.

Studies of the polyhedrosis role in population dynamics of gypsy moth, *H. cunea* and some noctuides demonstrated that the bulk of virus-infected insects died at the great ages (4^{th}-6^{th} instar). The maximum death rate of insects from a mixed infection was demonstrated with NPV and GV on *H. cunea.* Based on the mixed infection between NPV and GV, the production processes and application of the Virin-ABB-3 viral preparation have been developed, demonstrating high biological efficacy in the protection of orchards and forest crops.

Red Biotechnology

Health

Medical

Diagnostics

ANTIMICROBIAL ACTIVITY OF SOME COPPER (II) COORDINATION COMPOUNDS WITH N-(4- (2-((3-METHYL-5-OXO-1-PHENYL)-4,5-DIHYDRO-1H-PYRAZOL)METHYLENE) HIDRAZINCARBOTIOAMIDO) PHENYL)ACETAMIDE

Burduniuc Olga[1], Balan Greta[2], Rusnac Roman[3], Gulea Aurelian[3]

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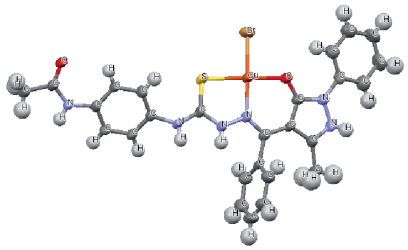
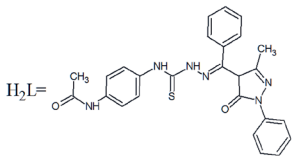
Coordination compounds based on heterocyclic tiosemicarbazones are reported more frequently in the literature demonstrating a broad spectrum of antimicrobial, antifungal and anticancer properties. Coordination compounds of copper (II) are increasingly being investigated on different biological properties, the structural investigation of some copper (II) compounds was performed by infrared spectroscopy, ^{1}H,^{13}C-NMR spectroscopy and single crystal X-ray diffraction.



This paper aims to elucidate the structure- antimicrobial activity relationship. The investigation of antimicrobial properties was performed on *Staphylococcus aureus* strains.

Table. Antimicrobial activity of some coordination compounds *MIC/MBC, mg/mL.**

Compound	*Staphylococcus aureus*	
	MIC	MBC
H_2L= 	> 0.2500	> 0.2500
$[Cu_2(HL)_2Cl_2]$	0.0625	0.1250
$[Cu_2(HL)_2Br_2]$	0.0625	0.1250
$\{Cu(L)H_2O\}$	0.1250	0.2500
$\{Cu(HL)NO_3\}$	> 0.2500	> 0.2500
$\{Cu(HL)ClO_4\}$	> 0.2500	> 0.2500

**MIC- minimum inhibitory concentration; **MBC-minimum bactericide concentration.*

Conclusion: The antimicrobial activity of studied copper (II) coordination compounds against *Staphylococcus aureus* was shown at minimal inhibitory concentrations of 0.0625–0.2500 mg/mL.

ANTIFUNGAL ACTIVITY OF SOME HETEROCYCLIC COMPOUNDS

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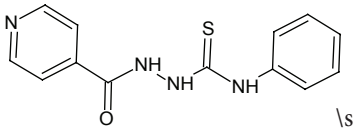
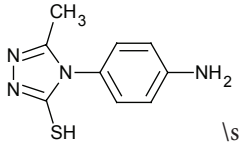
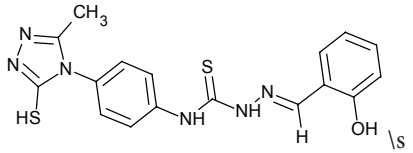
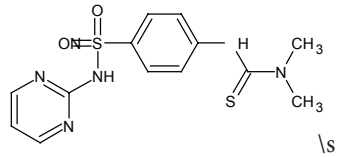
1,2,4-Triazole, pyridine and pyrimidine derivatives are more distinct from other heterocyclic compounds by their antimicrobial and antifungal properties, as well as reduced toxicity.

This paper aims to elucidate the structure-activity relationship. The investigation of antifungal properties was performed on *Candida albicans* strains. The reference substance was Nistatine, used in medicine to treat candidiasis.

Table. Antifungal activity of some heterocyclic compounds.

Compound	***Candida albicans***	
	MIC* µg/mL	MFC** µg/mL
 N-Phenyl-2-(pyridine-4-carbonyl)hydrazine-1-carbothioamide	31.25	62.50
 4-(4-Aminophenyl)-5-methyl-4*H*-1,2,4-triazole-3-thiol	125.00	250.00
 2-[(2-Hydroxyphenyl)methylidene]-N-[4-(3-methyl-5-sulfanyl-4*H*-1,2,4-triazol-4-yl)phenyl]hydrazine-1-carbothioamide	31.25	62.50
 4-[(Dimethylcarbamothioyl)amino]-N-(pyrimidin-2-yl)benzene-1-sulfonamide	31.25	125.00
Nystatin	80.00	80.00

**MIC- minimum inhibitory concentration; **MFC-minimum fungicide concentration.*

Conclusion: The studied compounds demonstrate antifungal activity at the reference level or even 2.5 times higher than Nystatin.

BIOMARKERS OF THE OXIDATIVE STRESS IN DIFFERENT FORMS OF TUBERCULOSIS

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Tuberculosis evolution and treatment response are determined by the mycobacteria virulence, and the organism's capacity to fight against the aggression of the oxidative stress through the antioxidant defense. The oxidative stress (OS) is caused by the imbalance between the systemic manifestation of the reactive oxygen species (ROS) and the ability of biological system to detoxify them and to repair the resulting damage. The disturbances in the normal redox system cause toxic effects through the peroxidation of the cellular DNA, proteins, lipids, carbohydrates and other biological macromolecules. Several biomarkers are available in the assessment of the OS. Advanced oxidation protein products (AOPP) are novel biomarkers, resulted from the interaction between the chlorine oxidants (chloramines and hypochlorous acid) with plasmatic proteins. AOPP are carried by oxidized plasma proteins, especially albumin, are excreted by the kidneys and the highest concentration were identified in patients with severe chronic renal failure, hyperparathyroidism and continuous treatment with calcium and vitamin D. Interleukin 8 (IL-8) is secreted by the macrophages, phagocytes and mesenchymal cells. It activates neutrophil chemotaxis and accumulation of the leukocytes at the site of the infection, inflammation, ischemia and traumatism. IL-8 releasing is induced by the IL-1 and TNF-α.

The aim of the research was to assess the correlation between the concentration of the IL-8 and oxidative biomarkers: advanced oxidation protein products, ureea, creatinine.

Material and methods: a prospective study which included 46 patients, new cases with pulmonary tuberculosis (study group-SG) were investigated according to the national protocol and compared with results of the 36 healthy persons (control group-CG). The determination of the AOPP was performed according to the modified method of Witko-Sarsat V [1]. The concentration of the IL-8, urea and serum creatinine was assessed through the spectrophotometric analysis using the kits of the producer Eliteh (France) according to the attached instructions.

Results: Distribution of patients, according to the biological characteristics established a similar rate of men in both groups, with their predomination 31 (67,39±6,91%) in SG vs. 24 (66,78±7,87%) in CG, which ensured the comparability of the groups. The concentration of the AOPP in the serum was statistically higher in the SG 44,06±2,86 μMol/l compared with the CG 34,349±3,58 μMol/l ($p<0,05$). The concentration of the blood urea was statistically higher in the SG 18,92±9,2 mg/dL compared with the CG 13±2,28 mg/dL ($p<0,05$), as well of the creatinine 79,79±6,84 mg/dL vs 45,87±5,69 mg/dL ($p<0,001$). The concentration of the IL-8 was eleven times higher in the SG 15,595±8,411 ng/ml in the SG than in the CG 1,163±1,685 ng/ml ($p<0,001$). The correlation between the serum concentration of the AOPP and IL-8 was assessed as a middle degree ($r=0,29$), the same degree was established for the correlation between IL-8 and ureea ($r=0,32$) and low degree between IL-8 and creatinine ($r=0,16$).

Conclusion: the concentration of the proinflammator biomarker IL-8 was significantly correlated with protein oxidation products: AOPP, urea and creatinine, which demonstrated severe proteic catabolism in patients with active tuberculosis.

Reference: 1. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int. 1996 May;49(5):1304-1313.

AZACHALCONE DERIVATIVES AND THEIR ANTIFUNGAL ACTIVITY

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This paper relates the synthesis and study of the antifungal activity of azachalcone derivatives (I) and (II). The synthesis of azachalcone (1,3-di(pyridin-2-yl)prop-2-en-1-one) by classical method did not allow to obtain the desired product. Condensation of 2-acetylpyridine with sodium carbonate-catalyzed 2-formylpyridine in aqueous medium has been shown to be effective to produce azachalcone. The products I and II were obtained by performing the condensation reaction both in the basic catalysis and in the presence of HCl(c.) in alcohol. The mechanism of formation of the product (I): the Claisen-Schmitd condensation and Michael addition generates the diketone intermediate, which participate in the aldol double reaction with the third molecule of 2-acetylpyridine (Fig. 1, I). The mechanism of formation of the product (II) from azachalcon and the diketone intermediate present in the reaction medium was confirmed by direct synthesis of the product from the starting substances obtained subsequently as a result of Michael addition, followed by intramolecular condensation and subsequent cyclisation (Fig. 1, II). For the characterization of the final and intermediate products FTIR, ^{1}H-NMR, ^{13}C-NMR spectroscopy were used. The structure of compounds I and II was confirmed by X-ray diffraction on the monocrystal (Fig. 1, I and II).

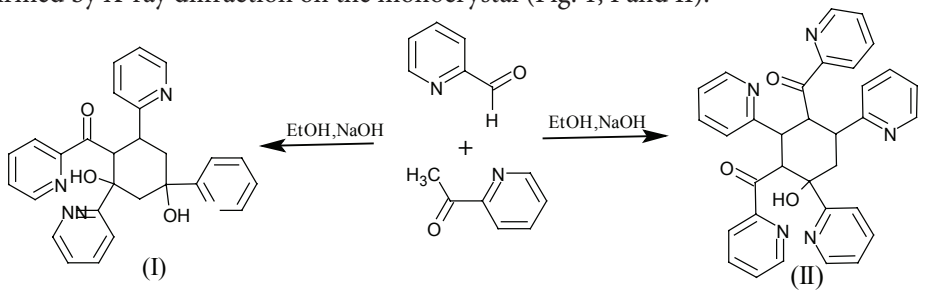

Fig.1. Condensation reactions between 2-acetylpyridine and 2-formylpyridine, which complete with the formation of the cyclohexane ring in compounds (I) and (II). I- structural formula of [2,4-dihydroxy-2,4,6-tri(pyridin-2-yl)cyclohexyl](pyridin-2-yl)methanone], II- structural formula of [4-hydroxy-2,4,6-tri(pyridin-2-yl)cyclohexane-1,3-diyl]bis(pyridin-2-yl)methanone].

Table. Antifungal activity of compounds (I) and (II) MIC*, MFC, mg/mL.**

Compound	Candida albicans		Candida krusei		*Candida parapsilosis*		*Cryptococcus neoformans*	
	MIC	MCB	MIC	MCB	MIC	MCB	MIC	MCB
I	0,50	1,00	0,50	1,00	0,25	0,50	0,1250	0,2500
II	0,25	0,50	0,25	0,50	0,25	0,50	0,0039	0,0078

**MIC- minimum inhibitory concentration; **MFC-minimum fungicide concentration.*

Conclusion: For compound (I) the minimal bacteriostatic and fungicidal concentration are within the limits 0.125-1.0 mg/mL and for compound (II) the minimal bacteriostatic and fungicidal concentration are in the range 0.0039-0.5 mg/mL.

THE INFLUENCE OF IMMUNOMODULATORS ON THE CONTENT OF PRO- AND ANTI- INFLAMMATORY CYTOKINES IN PATIENTS WITH PULMONARY TUBERCULOSIS.

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The interest of researchers in the cytokine system is explained by the key role of these molecules in the development of pathological processes. They perform the function of mediators, messengers of intercellular interactions; regulate the intensity and the type of immune response.

Objective of our research was to study the dynamics of pro-inflammatory and anti-inflammatory cytokines in patients with pulmonary tuberculosis under the influence of immunomodulators.

Material and methods. The study included 57 patients with pulmonary tuberculosis with anti-TB drugs sensitivity. The first group (38 patients) received adaptogen BioR simultaneously with antituberculosis drugs. The second group consisted of 19 patients who, for various reasons, received only anti-tuberculosis treatment. The content of IL-2 and IL-4 were examined by solid-based enzyme-linked immunosorbent assay-ELISA using reagents from OOO "Вектор–БЕСТ" (Russia).

Discussion. The content of pro-inflammatory cytokine IL-2 before the start of treatment was significantly higher than in the healthy patients in both groups of patients ($p < 0.001$). In dynamics, the content of IL-2 significantly decreased both in the first ($p < 0.001$), and in the second group ($p<0.01$), but in the second group this change was less pronounced. This is confirmed by the fact that the content of IL-2 in the second group after the treatment was significantly higher than in the first group ($p<0.01$). In the both groups of patients on admission there was approximately the same content of pro-inflammatory cytokines (without significant difference). In the dynamics of patients of the first group (where patients received BioR), a positive decrease in pro-inflammatory cytokines was noted, in the second group (where patients took only anti-tuberculosis treatment), only the tendency to decrease was noted. Such slow dynamics of pro-inflammatory cytokines IL-2 in the second group can lead to the chronic inflammation.

Table. The dynamics of the content of pro- and anti-inflammatory cytokines in the patients of the examined groups.

Index	IL–4	IL–2
Healthy	6,3±0,32	3,9±0,19
1 group (before)	6,2±0,55	9,5±1,06□
1 group (after)	10,7±0,92●	5,2±0,60●
2 group (before)	8,5±1,12	11,3±1,09□
2 group (after)	17,9±3,00○●	10,0±1,28○●

Note: □ significant difference between healthy and sick persons ● – significant difference between before and after the treatment ○ – significant difference between group 1 and 2 after the treatment

The content of anti-inflammatory cytokine IL-4 before the start of the treatment was approximately at the same level as in healthy and without significant differences (Table 1) in the both group of patients. In dynamics, IL-4 content significantly increased both in the first ($p < 0.001$), and in the second group ($p < 0.01$), but in the second group this change was less pronounced. This is confirmed by the fact that the content of IL-4 in the second group after treatment was significantly higher than in the first group ($p < 0.01$).

Conclusion. Thus, the use of the adaptogen BioR in combination with anti-tuberculosis treatment improves the ratio of pro- and anti-inflammatory cytokines and increases the patient's ability to adapt. In the group of patients who took only anti-tuberculosis treatment, on the contrary, the ratio of pro- and anti-inflammatory cytokines become unfavourable and the ability to adapt decreases.

STUDY OF BIOR PREPARATION ACTION ON FUNCTIONAL ACTIVITY AND ENZYMATIC SYSTEM OF LIMFOCITES

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The tendency to increase the number of patients with *Mycobacterium tuberculosis* resistant to specific antibacterial drugs persists. An unfavorable factor is the increase in multidrug resistance and polyresistance. Some authors showed the role of T cell immunity defect in pulmonary tuberculosis.

All components of immune cell metabolism (energy metabolism, synthesis of substances, use of metabolic products) are linked and coordinated with each other and are regulated by programs developed during the evolution of the organism that give the biochemical processes the necessary direction. Therefore, normalization of the immune system condition includes a mandatory component - normalizing metabolic disorders of immune cells.

The purpose of the research was to study the action of the BioR preparation on functional activity and the enzymatic system of lymphocytes.

Methods and object of research. According to the method of randomization and double-blind investigation, the following groups of patients were constituted:

I group, 48 patients - antituberculous treatment and BioR capsule preparation (BioR);

II group, 21 patients – antituberculosis treatment and placebo (placebo);

III group, 48 patients - anti-tuberculosis treatment (Anti-TB).

In the supernatant solution after lymphocyte culture, the lactate-dehidrogenase level was estimated. The reaction of blast transformation of lymphocytes (RBTL) with phycohemagglutinin (PHA) was used for evaluation of functional lymphocyte activity.

Results and discussions. Upon admission, functional lymphocyte activity in all groups was lowered with the same intensity and did not significantly differ. After treatment, the functional activity of lymphocytes in all groups increased, with the exception of patients who received antituberculous treatment

Table. Functional activity of lymphocytes in the groups of patients before and after the treatment (in %).

Indices		Healthy	BioR	Placebo	Anti-TBC
RBTL+PHA	until	79,9±1,16	61,8±1,21	58,6±2,09	60,8±1,27
	after		70,9±0,77◊	64,1±1,69◊●	64,2±1,22*
RBTL+PHA+BioR	until		67,2±0,82	65,2±1,44	65,2±0,95
	after		67,9±0,89	67,4±0,89	66,3±0,97
Lactate-dehydrogenase	until	167±5,7	151±6,7	128±7,2●	134±5,9
	after		181±7,2◊	143±7,8●	147±6,9*

*Note: the veracity of: ● - BioR and placebo; * - BioR and Anti-TBC.*
+ - placebo and anti-TB; ◊ Indications for admission and discharge.

The results of RBTL+PHA + BioR demonstrate an immunocorrection index with the same values in patients of all groups. After treatment, in a higher lymphocyte functional activity, the immunocorrection score in groups decreased (except for placebo), the reduction was conclusive for patients who received BioR. Changes in the placebo and anti-tuberculosis groups were less pronounced than those after BioR treatment. Thus, on the functional activity of lymphocytes, a more pronounced immunocorrection effect is manifested by BioR.

In the process of lymphocyte proliferation, there is an increase in lactate dehydrogenase (LDH) content, which regulates the intensity of anaerobic and aerobic cell energy processes, reflecting the particularities of metabolism and the functional specificity of cells and tissues. Hence the use of this enzyme as an indicator of functional state of cells, allows a more complete evaluation of lymphocyte functional activity. The LDH content at hospitalization was lower in patients in comparison with the content of this enzyme in the healthy group. After treatment, the lymphocyte content of LDH increased in all groups, more conclusive for patients who received BioR ($p<0.01$).

Conclusion. Thus, BioR preparation, according to the mechanism of action, manifests as an immunocorrector on the immunological reactivity of the organism and exhibits a pronounced immunocorrective activity on the functional and enzymatic lymphocyte system.

CHANGES OF THE ENDOGENOUS INTOXICATION INDICES IN PATIENTS WITH PULMONARY TUBERCULOSIS UNDER THE INFLUENCE OF IMMUNOMODULATORS

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Recently, a lot of scientific work has been devoted to the study of endogenous intoxication in various pathological diseases and conditions. Endogenous intoxication is one of the most important criteria determining the severity of a person condition (Разнатовская Е.Н., 2012). In this regard, the use of drugs in order to detoxify the body and normalize the changed immune status of patients has a particular importance in modern complex therapy of tuberculosis.

The aim of the work was to study the indices of endogenous intoxication in patients with pulmonary tuberculosis under the influence of immunomodulators.

Material and methods. The study included 57 patients with pulmonary tuberculosis with sensitivity to anti-TB drugs. The first group (38 patients) received concurrently BioR with anti-TB drugs. The second group consisted of 19 patients who, for various reasons, received only anti-tuberculosis treatment. Indices of endogenous intoxication were determined by the new method proposed by authors (Ghinda S. şi coaut. 2015).

Analysis and discussion of the results. The content of circulating immune complexes (CICs) was not the same for patients of different groups (see the table). The content of the CICs with high molecular weight (PEG 2.5%) and having low toxicity in patients of both groups was not significantly different on admission and was significantly lower ($p<0.001$ in both groups) than in healthy ones. After treatment, the content of the CICs with a high molecular weight (PEG 2.5%) decreased, but only in the first group this decrease was significant ($p <0.01$).

Table. Indices of endogenous intoxication (M ± m)

Indicators		Healthy n=100	1 (n=38)		2 (n=19)	
			before	after	before	after
PEG-2,5%	u.d.o.	7,2±0,35	15,2±1,96•	8,8± 0,86◊	24,2±4,36•	14,5±3,78
PEG-4,2%	u.d.o.	25,2±0,84	42,6±3,60•	31,7±3,56◊	53,9±5,53•	37,3±5,20◊
PEG-8,0%	u.d.o.	245±7,2	418±25,2•	293±18,9◊	460±42,3•	367±37,6

Note: • - significant difference between healthy and sick
◊ - significant difference between the indicators in the groups before and after treatment

The content of the CICs with an average molecular weight (PEG 4.2%), which had the greatest toxicity, was not significantly different in admission in patients of both groups and was significantly less ($p<0.001$ in both groups) than in healthy ones. After treatment, the content of the CICs with an average molecular weight (PEG 4.2%) was reliably reduced, with approximately the same intensity in both groups ($p<0.05$).

The content of the CICs with a small molecular weight (PEG 8.0%) and high toxicity in patients of both groups was not significantly different on admission and was significantly less ($p <0.001$ in both groups) than in healthy ones. The content of CICs with an average molecular weight (PEG 4.2%) decreased after treatment, but only in the first group this decrease was significant ($p<0.01$).

Conclusion: The use of the BioR preparation in the complex treatment of tuberculosis, with adaptogenic, antioxidant, immunoregulating and detoxifying properties, makes it possible to combat the syndrome of endogenous intoxication more effectively.

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NATURAL PRODUCT WITH APHRODISIAC-LIKE EFFECT CONTAINING SPIRULINA EXTRACT

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Sexual dysfunctions present a serious medical and social symptom that occurs in 10-52% of men and 25-63% of women. Organic causes of erectile dysfunction include hypogonadism, hyperprolactinaemia, and neurological disorders. Of the social causes of these disorders, the most important factor is stress. In addition, many treatments administered in case of diabetes mellitus, hypertension, and psychosis also contribute to worsening the situation with erectile dysfunction in men and libido in women. It is well known that people who suffer from stress and insomnia for a long time, their libido decreases, leading again to the onset of stress and insomnia, thus triggering a vicious circle of pathology.

Currently available drugs and treatments intended for sexual dysfunctions, including erectile ones, are limited in effectiveness, are characterized by numerous unpleasant side effects and contra-indications in certain disorders. Sildenafil citrate (Viagra) is one of the most successful drugs for this purpose, which alters penile hemodynamic, causing common side effects such as headaches, facial flushing or redness, dyspepsia and nasal congestion.

For these reasons, the development of effective new drugs with minimal adverse effects in the treatment of sexual dysfunction remains permanently the focus of researchers. Elaboration of preventive and therapeutic remedies based on natural aphrodisiacs is a perspective direction in the contemporary biomedicine. Starting from this, our researches have been focused on devising a remedy that, along with the antidepressant effect, also has aphrodisiac action. The selected form was a balm based on native vegetal raw material.

It was elaborated a balm with aphrodisiac-like effect, containing macerate obtained by maceration of vegetable raw material in hydroalcoholic solution in a ratio of 1:3, respectively, in the following ratio of components, g: licorice root 1.5 - 2.5, sweet calamus rhizomes 0.10 - 0.16, aboveground parts of St. John's wort 0.60 - 0.70, aerial parts of marjoram 0.49 - 0.55, peppermint leaves 0.78 - 0.84, aboveground parts of milfoil 0.70 - 0.80, pine buds 0.8 - 1.2, , acacia flowers 1.5 - 2.5, celery rhizomes with the aboveground part of celery 4.5 - 5.5, parsley root with the aboveground part of parsley 1.5 - 2.5. The balsam also contains hydroalcoholic extract of *Spirulina platensis* biomass of 20 mg/ml with a strength of 40 - 55% vol. 0.05 - 0.1 ml, hydroalcoholic extract of grape seeds of 0.05 mg/L with strength of 5 - 10% vol. 1.5 - 2.5 ml, alcoholic extract of walnut shells and septa of 10 mg/ml with strength of 45 - 55% vol. 0.05 - 0.1 ml, dessert red wine with strength of 16% vol. 300 - 400 ml, ethyl alcohol of 96% 285 - 289 ml and purified water up to 1000 ml. By developing this balm, we have expanded assortment of balms possessing high biological activity. The product priorities are the improvement of organoleptic properties due to the introduction of new ingredients and the significant increase in libido. This was also achieved due to selection of quantitative and qualitative ratio of balm components, their unique properties and synergistic effect manifested in mixing them.

This balm was applied to 32 patients who displayed clinical signs of decrease in libido. Actually, 29 of them experienced obvious changes to increase testosterone levels in the blood, an average of 3.6 nmol/L, within 60 days towards data up to the use of balm.

Thus, product peculiarities allow for an effective increase in the amount of testosterone and libido in people suffering from stress and insomnia, and the removal of the pathological vicious circle between stress and libido.

THE INFLUENCE OF IMMUNOMODULATORY THERAPY ON T-LYMPHOCYTES AND SOME INDICATORS OF THEIR METABOLISM.

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The inclusion of immunomodulators into the pathogenetic therapy increases the susceptibility of patients to the various regimens and its effectiveness. It was established that the suppression of energy metabolism in lymphocytic cells entails the aggregation and lysis of lysosomes, the release of their contents, and as a consequence, its apoptosis.

The **purpose** of our research was a comparative study of the effect of the drugs BioR and Mellozan on the dynamics of certain indicators of the metabolism of T- lymphocytes in patients with resistant forms of pulmonary tuberculosis under the influence of immunomodulators.

Materials and methods. The study included 112 patients with multi drug (MDR) - resistant forms of pulmonary tuberculosis: 1 – the control group - 31 patients who received antituberculous treatment (ATT); 2 – the experimental group - 31 patients who received ATT and Mellozan, 3 – the experimental group - 50 patients who received ATT and BioR. To evaluate the T- lymphocyte count were used the reaction of blast transformation of lymphocytes (RBTL) and CD3 lymphocyte counting using Flow Cytomertrie (Partec PAS I) cytofluorometer. In the supernatant, after cultivation of lymphocytes, the glucose content was determined.

Analysis and discussion of the results. The content of lymphocytes (see the table) at admission was approximately at the same level in all three groups of patients and significantly less than in healthy patients ($p<0.001$). Under the influence of the studied drugs, the lymphocyte content was raised in all groups of patients - less active in group 1 ($p<0.05$), with the same activity in groups 2 and 3 ($p<0.001$). However, the increase in lymphocyte counts in the 2nd group was more pronounced and reached a significant difference between the 1 and 2 group after treatment ($p<0.01$). The functional activity of lymphocytes (RBTL+PHA) at admission was approximately at the same level in all three groups of patients and significantly less than in healthy patients ($p<0.001$). Under the influence of the studied drugs, the functional activity of lymphocytes was elevated in all groups of patients - less active in group 1 ($p<0.05$), with the same activity in groups 2 and 3 ($p<0.001$). However, the increase in the functional activity of lymphocytes in group 3 was more pronounced and reached a significant difference between groups 1 and 3 after treatment ($p<0.05$).

Table. Characteristics of lymphocytes CD3, of RBTL+PHA and of glucose consumption (GC)

Index		Healthy (n-50)	1group (n–31)	2group (n–31)	3 group (n–50)
Lymphocytes CD3 (%)	before	67,4±0,53	52,3±0,83	52,8±0,92	51,5±0,74
	after		55,0±0,86▪	58,7±1,00▪	56,4±0,67
RBTL + PHA (%)	before	79,9±1,16	56,5±1,06	56,5±1,15	56,1±1,44
	after		60,1±1,22▪	62,8±1,03▪	63,9±1,22▪
GC (mmol/l)	before	4,7±0,20	1,77±0,113	1,55±0,062	1,51±0,081
	after		2,09±0,113	1,78±0,077▪	1,83±0,108▪

Note. The significant difference between the indicators: ▪ before and after treatment

The glucose consumption (GC) from the lymphocyte culture medium in all three groups was at the same level, without significant differences and statistically significantly reduced compared to the healthy group ($p<0.001$ in all patient groups). Under the influence of the studied drugs, glucose consumption from the lymphocyte culture medium was raised in all groups of patients - less active in group 1, with the same activity in groups 2 and 3 ($p<0.05$ in both cases).

The conclusion. The drug BioR has a more pronounced stimulating effect on the indices of the functional activity of T-lymphocytes(CD3), and Mellozan has a more pronounced stimulating effect on T- lymphocyte(CD3) counts. Both drugs had the same stimulating effect on the glucose consumption.

THE *IN VITRO* EFFECT OF THE *EN-* PREPARATION ON THE PRE-IMMUNE RESISTANCE INDICES IN PATIENTS WITH CHRONIC TONSILLITIS.

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Polyphenols (isolated from plants) are one of the types of antioxidants that protect cells of the human body from harmful reactions. Polyphenols are not only antioxidants, but also probiotics, i.e. they suppress the growth of the pathogenic microflora of the digestive tract, thereby promoting the vital activity of beneficial bacteria. The ongoing differentiation and specialization of immunology branches raise the today`s urgent problems - the need to develop a new direction that emerged at the junction of immunology, phytoimmunology and biotechnology – ***immune-biotechnology***.

The preparation EN is of interest, being prepared from walnut, which contains polyphenols up to 20-22%, free amino acids up to 0.92-1.02% (of which essential ones are up to 0.17-0.20%, immunoactive ones being up to 0.20 -0.22%), iodine - up to 0.2 mg/l and iron - up to 0.4 mg / l. In this regard, we set the **goal to investigate** the *in vitro* effect of the EN preparation on neutrophil activity by nitroblue tetrazoliun (NBT) test.

Material and methods. The study included 39 patients with chronic tonsillitis. The object of study was patients` neutrophils. A standard NBT test was performed, a suspension of leukocytes was poured into the control holes, while a leukocyte suspension and EN preparation diluted 1/128 was poured in the test holes.

Discussion of the results.

The NBT-test in the group of healthy children was 0.12 ± 0.003, which is significantly higher than in the 1 subgroup of patients with the stimulating effect ($p<0.001$) and significantly less than in the 3 subgroup of patients with a suppressive effect ($p< 0.001$). The NBT-test in the 2 subgroup of patients, where no modulating effect was found, was the same as in the healthy group. The NBT-test in the 1 subgroup, where the stimulating effect was noted, was initially significantly lower than in the 2 and 3 subgroups ($p<0.001$ in both cases), and under the influence of the EN preparation it increased to the level of healthy ones.

Table. Analysis of NBT-test indices in subgroups of patients

Index	**EN (39 patients)**		
	1 (stimulating) 23 (60%) patients	**2 (without modulation) 10 (25%) patients**	**3 (suppressive) 6 (15%) patients**
NBT-test (healthy)	0,12±0,003		
NBT-test (control)	0,09±0,001■	0,12±0,002○	0,14±0,004□
NBT-test (EN)	0,12±0,002■	0,12±0,002○	0,11±0,003□
ИМ	1,26±0,032■	1,0±0,00○	0,81±0,0322□

■ - *reliability between 1–2 subgroups,* □ - *reliability between 1–3 subgroups,*
○ - *reliability between 2–3 subgroups*

The NBT-test in group 3, where the suppressive effect was noted, was initially significantly higher than in the 1 and 2 subgroups ($p<0.001$ in both cases), and under the influence of the EN preparation it decreased to the level not significantly different from the healthy ones. Thus, the EN preparation does not have a modulating effect on the NBT-test parameters close to those of healthy ones. EN has a stimulating effect on the NBT-test which is lower than in healthy people, and EN has a suppressive effect on the NBT-test which is higher than in healthy ones.

Conclusion: the EN preparation showed a multidirectional effect; the EN preparation acted in a stimulating manner on low indices, in a suppressive manner on high indices and on indices close to normal - did not have any modulating effect. The EN preparation can be used to modulate the preimmune resistance indices in children with chronic tonsillitis aged 15 years and older.

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITIES OF IRON (III), COBALT (III), NICKEL (II) AND ZINC (II) COORDINATION COMPOUNDS WITH 2,4-DIHYDROXYACETOPHENONE 4-ALLYLTHIOSEMICARBAZONE

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From literature sources it is known that many thiosemicarbazide derivatives and their biometal coordination compounds possess antimicrobial, antituberculous, antifungal, antitumor and other kind of biological properties. Therefore, accumulation and systematization of the experimental data on synthesis and properties of new coordination compounds of biometals with thiosemicarbazide derivatives remains actual nowadays. Iron (III), cobalt (II), nickel (II), and zinc (II) salts react with 2,4-dihydroxyacetophenone 4-allylthiosemicarbazone (H_2L) forming coordination compounds with composition: $M(HL)_2X$ ($M=Fe^{3+}$; Co^{3+}; $X=Cl^-$; NO_3^-); $Ni(HL)X{\cdot}nH_2O$ ($X=Cl^-$; NO_3^-; n=2-3), $Zn(H_2O)(L)$.

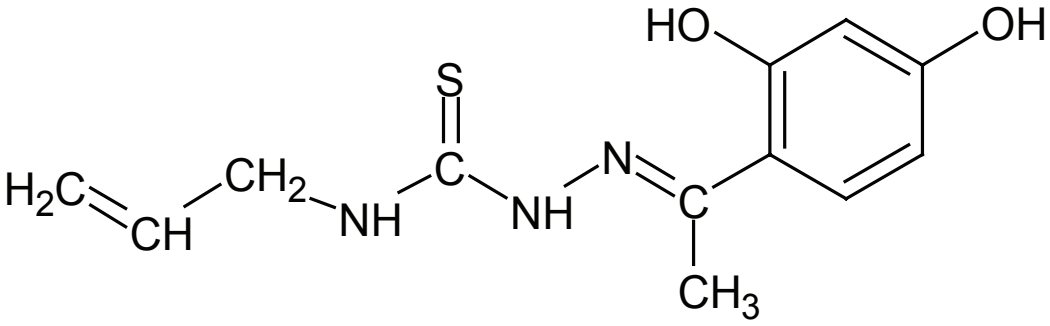

Fig. H_2L - 2,4-dihydroxyacetophenone 4-allylthiosemicarbazone

The antimicrobial and antifungal activities of these compounds were studied *in vitro* using the method of two-fold serial dilutions in liquid nutrient medium (meat infusion broth, pH = 7.0). The substances were dissolved in DMSO in such amounts that the solutions of concentration 10 mg / mL were obtained. The next dilutions were made using meat infusion broth. The antimicrobial activity was tested on standard strains of gram-positive (*Staphylococcus aureus* ATCC 25923), gram-negative *(Escherihia coli* ATCC 25922) microorganisms. The antifungal actitivity was studied on *Candida albicans.*

Table. Antimicrobial activity of synthesized coordination compounds

Compound	*Staphylococcus aureus*		*Eschirichia coli*		*Candida albicans*	
	MIC μg / mL	MBC μg / mL	MIC μg / mL	MBC μg / mL	MIC μg / mL	MFC μg / mL
H_2L	31.25	62.5	-	-	500	-
$Fe(HL)_2NO_3$	250	500	-	-	31.25	62.5
$Co(HL)_2NO_3$	250	500	-	-	125	250
$Co(HL)_2Cl$	250	500	-	-	-	-
$Ni(HL)NO_3 \cdot 3H_2O$	3.91	7.81	-	-	62.5	125
$Ni(HL)Cl \cdot 2H_2O$	7.81	15.63	-	-	62.5	125
$Zn(H_2O)(L)$	15.63	31.25	250	500	125	250

MIC – minimum inhibitory concentration, MBC- minimum bactericidal concentration, MFC-minimum fungicidal concentration

It was determined that H_2L thiosemicarbazone manifests antimicrobial activity towards only gram-positive microorganisms and a weak inhibitory activity towards *Candida albicans* at 500 μg / mL concentration. The nature of the central atom has the main influence on antimicrobial and antifungal activity of the synthesized coordination compounds. The antimicrobial activity of cobalt (III) and iron (III) coordination compounds is lower than of the thiosemicarbazone H_2L. Zinc (II) and nickel (II) possess higher activity than the corresponding pro-ligand. Iron (III) coordination compound manifests the highest antifungal activity. All substances except zinc (II) coordination compound manifest neither inhibitory nor bactericidal activity towards gram-negative microorganisms in the studied concentration range.

This work was fulfilled with the financial support of the Project 18.80.07.17A/PS of the State Program.

THE ROLE OF THE CO-MORBID CONDITIONS: DRUG DEPENDENCE AND OTHER ASSOCIATIONS ON TUBERCULOSIS TREATMENT OUTCOME

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Drug injection is considered an important issue for the public health of the Republic of Moldova (RM), due to the associated criminality and high rate of social determinated comorbidities. In the period of 2012-2013 a total number of 19400 intravenous drug users (IVDU) were estimated on the left side of the RM and 10800 on the right side. Due to the globalisation of the drug use the development of the legislation that targets the disease prevention due to unsafe drug use, increasing the accessibility to counseling and substitutive treatment was performed. In the RM an important issue is the long duration/systematic use of such drugs: opioids, cocaine and amphetamine. In the EU states the most used drugs are the heroine and other opioids.

The drug users are the key population for HIV infection, B and C hepatitis, TB and sexual transmitted diseases. Among Moldovan young adults (18-15 years) the rate of HIV was 8,5%, viral hepatitis 9,1%, TB 1,24% and sexual transmitted infection 12,7% in 2013. The socioeconomic vulnerability of the drug users is due to excessive consumption of drugs, alcohol, unemployment, single civil state, high rate of infractions and detention history. The associated epidemiological threaten due to high TB burden, put IVDU in a continuous danger to acquire infection and get sick.

The main **objective** of the research was to identify the particularities of IVDU with TB and to establish their final treatment outcome.

Material and methods: The study methodology had a quantitative and a qualitative component. The clinical study was performed as a retrospective, randomized control study, selective, descriptive research targeting risk factors, microbiological and radiological peculiarities, and treatment outcome of a total number of 267 patients, divided in three groups. The study group (SG) which included 48 TB patients with drug use, the first control group (1st CG) included 34 drug users and the second control group (2nd CG) included 185 new cases with pulmonary TB. Including criteria were: age >18 years, pulmonary TB and drug use were diagnosed by the specialists; in the 1st CG patients were diagnosed by the specialists from the Republican Dispensary of the Narcology.

Results: Distributing patients according to the sex, it was established the statistical predominance of men in all three groups, with the highest rate in the 1st CG. So, men were 33 (97,1%) in the 1st CG, 42 (87,5%) in the SG and 138 (74,6%) in the 2nd CG, with a male/female ratio=7/1 in the SG, 33/1 in the 1st CG and 2,9/1 in the 2nd CG. The urban residence predominated in all three groups, with the highest rate in the 1st CG: 100% compared with 139 (75,3%) in the 2nd CG and 35 (72,9%) in the SG. Homeless patients were identified in a similar proportion in the SG and 2nd CG: 8 (16,7%) and 29 (15,6%) respectively. The rate of employed patients was high in the 2nd CG 10 (29,4%), compared with 25 (13,5%) in the 3rd SG and 2 (4,7%) in the SG. Unemployed patients statistically predominated in the SG 43 (89,6%) compared with the 23 (67,6%) in the 1st CG and 124 (67,1%) in the 2nd CG. Poor living conditions predominated in

the SG 29 (60,4%) and the 2nd CG 106 (57,3%) compared with the 1st CG 14 (41,2%). Migrants were established only in the 2nd CG 24 (12,9%) and SG 8 (16,7%). History of detention was established in the SG 16 (33,3%) and 1st CG 7 (20,6%). Close TB contact was established in the SG 15 (31,2%) and 2nd CG 15 (8,11%). The HIV infection statistically predominated in the SG 21 (43,7%) compared with 11 (5,9%) in the 2nd CG and 1 (2,9%) in the 1st CG. Viral hepatitis were established only in the 1st CG 6 (17,6%), as well mental disorders 13 (38,2%) compared with 4 (2,16%) in the 2nd CG and 1 (2,1%) in the SG.

The final anti TB treatment outcome was expressed through a high rate of lost to follow-up and died in the SG compared with the 2nd CG: 10 (20,8%) against 2 (1,08%) and 8 (16,7) against 11 (5,9%), as well a low success rate 21 (43,7%) against158 (85,4%).

Conclusions: Associated risk factors as socioeconomic vulnerability and comorbid state contributed to a poor outcome. Social support and awareness about the free of charge diagnosis and treatment among key populations must be increased for improving the epidemiological state of the Republic of Moldova.

ANTIMICROBIAL AND ANTIFUNGAL EFFECT OF SOME BIOMETAL COORDINATION COMPOUNDS WITH 2-[(3-METHYL-5-OXO-1-PHENYL-4,5-DIHYDRO-1*H*-PYRAZOL-4-YL)-(PHENYL)METHYLIDENE]HYDRAZINECARBOXIMIDAMIDE

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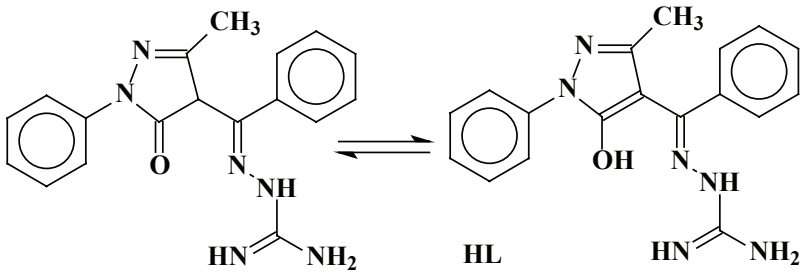
The aim of this work is the determination of antimicrobial and antifungal activities of manganese, iron, cobalt, nickel, and copper coordination compounds with 2-[(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)(phenyl)methylidene]hydrazinecarboximidamide (HL).

Chlorides, nitrates and acetates of stated above metals form coordination with the following composition: $M(L)_2X$ ($M = Fe^{3+}$, Co^{3+}, $X = Cl^-$, NO_3^-), ML_2 (M = Mn, Ni) and Cu(L)X($X = Cl^-$, NO_3^-, CH_3COO^-).



The antimicrobial activity of these compounds was studied *in vitro* using the method of two-fold serial dilutions in liquid nutrient medium (meat infusion broth, pH = 7.0). The substances were dissolved in DMSO in such amounts that the solutions of concentration 10 mg / mL were obtained. The next dilutions were made using meat infusion broth. The antimicrobial activity was tested on standard strains of gram-positive (*Staphylococcus aureus* ATCC 25923), gram-negative *(Escherihia coli* ATCC 25922) bacteria and *Candida albicans.* 18-Hours agar culture of these microorganisms was used for bacterial inoculation. Inoculation dose of 250-500 ths. microorganisms per 1mL was determined by optical turbidity standard. Test tubes were agitated and placed in thermostat at 37^0C for 24h. The test tube with nutrient medium and microorganisms but without the studied substance was used as control sample. If there were no signs of visible growth of microorganisms in the liquid nutrient medium then the tested substance was defined as bacteriostatic at this concentration. For detecting bactericidal activity of tested substance microorganisms were reinoculated on a solid nutrient medium (meat infusion agar) and incubated. The absence of visible growth of microorganisns indicates the bactericidal activity of tested substances at this concentration.

It was determined that the synthesized coordination compounds manifest selective bacteriostatic and bactericide activity towards both gram-positive and gram-negative microor-

ganisms in the range of concentrations 0.016-1.0 mg / mL. The experiment showed that the nature of the central atom and acid residue have the main influence on the minimal inhibitory (MIC) and bactericidal (MBC) concentrations of the studied coordination compounds. For the homotypic coordination compounds MICs and MBCs change in the following way: Co^{3+} ³ Ni^{2+} > Mn^{2+} > Cu^{2+} > Fe^{3+} and Cl^- > NO_3^- > Br^- > CH_3COO^-.

This work was fulfilled with the financial support of the Project 18.80.07.17A/PS of the State Program.

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF IRON(III), COBALT(III), NICKEL(II) AND COPPER(II) COORDINATION COMPOUNDS WITH 3,5-DIBROMOSALICYLALDEHYDE 4-ALLYL-S-METHYLISOTHIOSEMICARBAZONE

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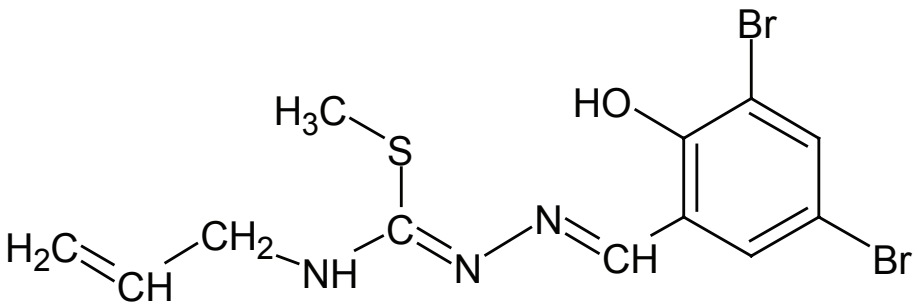
The search for novel biologically active substances with more selectivity and lower toxicity continues to be an area of intensive investigation. Isothiosemicarbazones have not been studied extensively from a biological point of view. The introduction of methyl group on the sulfur atom leads to a change of the method of coordination and biological properties.

Iron(III), cobalt(II), nickel(II), and copper(II) salts react with 3,5-dibromosalicylaldehyde 4-allyl-S-methylisothiosemicarbazone (HL) forming coordination compounds with composition: Cu(L)X (X=Cl^-, Br^-), $M(L)_2X$ (M=Fe^{3+}, Co^{3+}; X=I^-, NO_3^-, CH_3COO^-); $Ni(L)_2$.



HL - 3,5-dibromosalicylaldehyde
4-allyl-S-methylisothiosemicarbazone

The antibacterial and antifungal activities of these compounds were studied *in vitro* using the method of two-fold serial dilutions in liquid nutrient medium (meat infusion broth, pH = 7.0). The substances were dissolved in DMSO in such amounts that the solutions of concentration 10 mg / mL were obtained. The next dilutions were made using meat infusion broth. The antibacterial activity was tested on standard strains of gram-positive (*Staphylococcus aureus* ATCC 25923), gram-negative *(Escherichia coli* ATCC 25922) microorganisms. The antifungal actitivity was studied on *Candida albicans.*

Table. Antimicrobial activity of synthesized coordination compounds.

Compound	*Staphylococcus aureus*		*Escherichia coli*		*Candida albicans*	
	MIC mg / mL	MBC mg / mL	MIC mg / mL	MBC mg / mL	MIC mg / mL	MFC mg / mL
HL	0.0625	-	-	-	0.125	0.25
Cu(L)Br	0.000977	0.000977	-	-	0.0625	0.125
Cu(L)Cl	0.000977	0.000977	-	-	0.125	0.25
$Fe(L)_2NO_3$	0.5	-	-	-	0.25	0.5
$Co(L)_2NO_3$	0.0625	0.125	0.25	0.5	0.125	0.25
$Co(L)_2I$	0.125	0.25	0.25	0.5	0.125	0.25
$Co(L)_2(CH_3COO)$	-	-	-	-	0.5	-
$Ni(L)_2$	0.5	-	-	-	0.125	0.25

It was determined that HL isothiosemicarbazone manifests antifungal activity against *Candida albicans* and bacteriostatic activity towards only gram-positive bacteria. The nature of the central atom has the main influence on antimicrobial activity of the synthesized coordination compounds. The antibacterial activity against gram-positive microorganisms significantly growth in case of copper coordination compounds. Iron, cobalt and nickel coordination compounds have practically the same biological activity as the corresponding isothiosemicarbazone. Only cobalt coordination compounds manifest a weak antimicrobial activity against gram-negative microorganisms.

This work was fulfilled with the financial support of the Project 18.80.07.17A/PS of the State Program.

COMPARATIVE ANALYSIS OF IMMUNOMODULATORY THERAPY IN PATIENTS WITH TOXOCARIASIS ASSOCIATED WITH DISEASES OF THE RESPIRATORY ORGANS

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The adequate complex therapy of infectious diseases continues to evolve constantly. Effective treatment of these patients requires an algorithm that would influence the adaptive and protective functions of the organism through improved immune response. Suppression induced by parasitic antigens lead to the inhibition, proliferation and suppression of suppressive T lymphocytes, so that the entire process is self-controlling, by overcoming specific suppression.

The goal of this study: comparative analysis of immunomodulatory therapy in patients with toxocariasis associated with diseases of respiratory organs.

The materials and methods. Immunity was investigated in 83 patients with respiratory pathology-associated toxocariasis. In the supernatant solution after the cultivation of lymphocytes is determined the level of glucose. The patients, according to the non-randomized allocation method, were divided into three groups: the first group - 33 patients: anti-parasitic treatment Eskazol and BioR; second group - 28 patients: antiparasitic treatment Eskazol and Polioxidoniu; third group - 22 patients: antiparasitic therapy Eskazol.

Results and discussions. The number of lymphocytes in all groups examined prior to treatment was significantly higher than in healthy persons ($p < 0,001$). After treatment, the number of lymphocytes in the first and second group of patients increased significantly ($p<0,05$), and in the third group (without immunocorretion) only a tendency of quench increased. All this suggests a more favorable progression of the disease in groups 1 and 2 compared to group 3.

Due to the fact that the examined patients we determined a significant decrease in T lymphocytes (CD3) and the functional activity of T lymphocytes by blast transformation reaction with PHA, we wanted to analyze the metabolic parameters of the lymphocytes (see table).

The use of glucose (CG) by the T lymphocytes in the supernatant in all patient groups before treatment was verily low ($p<0,001$) compared to healthy persons. After treatment, this index continued to verily increase only to the patients in the first group ($p <0,001$), the patients in the 2 and 3 group there was only a tendency to increase it.

Table. The characteristics of glucose consumption (CS) indices, lactate dehydrogenase activity (LDG) and aldolase (ALD) of lymphocyte from the lymphocyte culture medium (M ± m)

Index		Healthy (n-50)	First group (n-32)	Second group (n-28)	Third group (n-22)
Lymphocytes (%)	up after	25,6±0,39	31,8±1,54 39,3±1,47▪	29,8±1,76 35,5±1,81▪	36,9±1,98 38,8±2,35
CG (mmol/l)	up after	4,7±0,20	2,12±0,081 2,42±0,107▪	2,22±0,108 2,51±0,0113	1,98±0,143 2,10±0,157

Significant difference between pointers: ▪ up and after treatment

Significant inhibition of T lymphocytes metabolism was determined in all the groups investigated prior to treatment, after treatment the modified indices increased more pronounced in the first and second group of patients, but did not reach the norm.

Conclusion. The use of specific antiparasitic therapy with immunocorrectors induces a positive dynamics of the pathological process, while the administration of only antiparasitic therapy does not provide a favorable evolution of the pathological picture. Significant inhibition of T lymphocyte metabolism has been determined in all investigated groups prior to treatment, after the treatment the modified indexes increased more pronounced in the first (BioR) and second group of patients (Polioxidoniu), but did not reach the norm.

SCREENING OF NEW STRAINS OF LACTIC ACID BACTERIA FROM KAZAKH TRADITIONAL FOOD PRODUCTS, PRODUCING A RECEPTOR FOR HUMAN PLASMINOGEN

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At present, the key role of the plasminogen-plasmin (Plg-Pl) system in homeostasis, chemotaxis, tissue remodeling, as well as proteolysis of the extracellular matrix and basal membrane, promoting cell migration in eukaryotes, has been established, both in physiological and pathological processes. The reception of plasminogen is known for pathogens more than fifteen years ago and only at the end of the last decade it was found that association with Plg is also possible in probiotics. Actual genomic and proteomic studies are aimed at finding genes and proteins of probiotic microorganisms that bind Plg and have a beneficial effect on human health.

It is known from scientific periodicals that the binding of plasminogen occurs via a receptor protein that can be localized both at the surface of the cell and be free in the extracellular fluid because of the physico-chemical properties of the receptor protein.

The aim of present study was the screening of lactic acid bacteria (LAB) from kazakh traditional food products for plasminogen binding activity.

9 new LABs from local traditional food products, as sources of new strains with probiotic properties were isolated and identified, using standard methods of microbiology and molecular biology.

The screening of LABs for plasminogen-binding activity, i.e. for the presence of the Plg receptor protein, was conducted using Western blot analysis. In this regard, binding (immobilization) of Plg on cells and binding to the target receptor protein in the cell-free supernatant fraction have been tested after centrifugation of the cell suspension. The cell pellet and the concentrated supernatant were mixed with SDS-buffer for Laemmli electrophoresis. After incubation at 100 ° C for 10 minutes and clarifying the samples by centrifuging 12,000 xg for 5 minutes, the samples were loaded to the wells of the gel and the electrophoresis in a PAA gel with SDS was performed. Next, the separated polypeptides from the gel were transferred to a nitrocellulose membrane using a Trans-Blot Electrophoretic Transfer Cell apparatus. After blocking nonspecific centers, the membrane was incubated with 0.5 μg/ml human plasminogen (Plg) (Sigma-Aldrich) in PBS overnight at 4 ° C. The [polypeptide * Plg] complex was detected by incubating a membrane with mouse anti-Plg antibodies and peroxidase-conjugated anti-IgG immunoglobulins as a secondary antibody.

At the moment 9 cultures are searched for plasminogen-binding activity. Based on the results of this phase of research, a new strain of LAB with plasminogen binding activity will be selected.

ANTIVIRAL POTENTIAL OF EXOPOLYSACCHARIDES PRODUCED BY LACTIC ACID BACTERIA

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Herpes viral infection is the most common human viral infection. It is known that lactic acid bacteria (LAB) can be used as a source of the substances with the diverse chemical structures and spectrum of biological activity for designing of products for medical purposes. Thus the screening of compounds with antiviral activity among such bacteria is still valid and has practical significance for the development of new antiherpetic drugs.

The aim of this work was to study the antiviral properties of 10 exopolysaccharides (EPSs) of lactic acid bacteria of the genera *Lactobacillus*, *Leuconostoc* and *Pediococcus*. Cytotoxicity and antiviral activity of EPSs were determined in cell culture MDBK by standard technique using MTT assay. The influence of EPSs on the herpes simplex virus 1 type (HSV-1) was determined by the virucidal, adsorption and penetration assays.

All EPSs demonstrated the minimal cytotoxicity of cells and their CC_{50} values were >2.7 mg/ml. It was determined, that EPSs exhibited *in vitro* anti-HSV-1 activities at different magnitudes of potency; their EC_{50} value equal to 0.2 and 1.1 mg/ml, and the selectivity index was in the range of 3 – 52.

To determine the stage of the HSV-1 inhibited infection, the virus and cells were treated with the compounds at various times before and after the HSV-1 infections. The exopolysaccharides showed virucidal activity and reduced the HSV-1 infectivity by 64 – 98% when were added to virus 3 h before adsorption. Our studies revealed that EPSs were able to prevent the HSV-1 attachment to cells and penetration into cells by 53 – 99% and 51 – 99 %, respectively.

When the compounds were added to cells at the end of the virus adsorption period (2 h after infection), a significant delay in the growth of HSV-1 was observed, and a much lower yield of infectious virus was also obtained. The EPS 6, EPS 8, EPS 9 and EPS 10 reduced HSV-1 virus production by 30 – 58%, 62 – 80%, 34 – 58% and 50 – 99%, respectively.

Thus, we showed that isolated exopolysaccharides (EPS) of lactic acid bacteria of the genera *Lactobacillus* and *Leuconostoc* possess the antiviral activity and cause inhibition of HSV-1 reproduction. The data indicate that the use of EPSs of lactic acid bacteria in creation of the new classes of drugs is a promising approach for the treatment of diseases caused by herpes simplex virus.

UTILITY OF SOME SPECIES OF INTESTINAL STREPTOCOCI FOR THE ORGANISM

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Analysis of existing information found streptococci belonging to the *Streptococcaceae* family which includes multiple genera, but only some of them are considered to be useful for the human and animal body. This is due to their biological properties (antagonistic properties, adhesion, biosynthetic activity etc.) This fact has been confirmed in our previous works, because all strains of streptococci isolated from the rectal content of young children and young animals have been identified as belonging to *Streptococcus*, *Lactococcus* and *Enterococcus* genera and the nominated properties were the basis for the selection of strains that can be used in the production of probiotic microbial preparations.

The purpose of this paper is to identify and to describe the useful characteristics of intestinal streptococci and their role in health.

That is why the experiments with intestinal streptococcal strains isolated for the first time from children, calves and piglets have been carried out with the purpose of studying their useful properties: antagonistic activity and adhesive capacity.

As a result of research it was found that all strains of intestinal streptococci have showed antagonistic properties, with different level of inhibition of test microorganisms (*Escherichia* and *Salmonella*). For instance, for specific to the digestive tract of children enterococci the inhibition level was within the limits 74,55%-82,60% against *Escherichia* and 72,35%-73,47% against *Salmonella* ; of calves – 82,35%-86,48% and respectively 64,55%-68,25%; of piglets - 81,39%-85,62% and respectively 63,54%-65,66%. At the same time, the antagonistic activity of *Streptococcus* strains was higher against *Escherichia* (85,45%-89,35%) and lower against *Salmonella* (66,45%-70,24%). The lowest level of inhibition was recorded for lactococci: 58,39%-70,37 against *Escherichia* and 54,50%-64,53% against *Salmonella*. This antagonistic activity was confirmed by their properties to synthesize bacteriocins.

Because the protective role of intestinal microbial representatives is due to their adhesive capacity, the adhesion index of isolated intestinal streptococci was determined. For the specific to digestive tract of children strain, this index was higher in the case of the *Enterococcus* genus (4,58-4,7 c.u.), followed by *Streptococcus* (3,66-4,37 c.u.) and *Lactococcus* (3,16-3,70 c.u.). In the case of streptococcal strains isolated from calves the adhesion index were found respectively within the limits of 4,38-4,50; 3,25-3,62 and 2,90-3,30; and from piglets - 4,28-4,66; 3,13 –3,46 and 2,62-3,04 u.c.

Therefore, based on the obtained results, the native strains of intestinal streptococci (of the genera *Lactococcus*, *Streptococcus* and *Enterococcus*), especially, *L. lactis, S. thermophilus* and *E. faecium* can be recommended for the use in the production of fermented dairy products for the children. They can be included in the composition of microbial preparations with differentiated probiotic action, because of their role in maintaining the sanogenic state of microbial digestion in the digestive tract. Such new products are strictly necessary for the dairy and pharmaceutical products for bacterioprophylaxis and bacteriotherapy of intestinal pathologies (dysmicrobism and diarrheal dysfunctions).

GLYCEROLE-OXIDAZING ENZYMES SYNTHESIZED BY FUNGI OF GENERA *ASPERGILLUS* AND *PENICILLIUM*

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Determination of glycerol concentration is an important task of analytical biotechnology. Glycerol is one of metabolites of alcoholic fermentation characterizing taste qualities of drinks and food products. It is used in manufacture of explosives, cosmetic and medicines. Determination of triglycerides and glycerol in human blood is a vital indicator of risk caused by of complications of cardiovascular diseases and systemic disorders of fat metabolism. Enzymatic methods for determination of glycerol are the most accurate and environmentally safe.

Taking into account the demand for simple and effective method to determine glycerol in various industries and medicine, the important task is to seek of new strains-producers of glycerol oxidizing enzymes and to develop perspective productive technologies.

Early screening of strains – potential producers of glycerol-oxidizing enzymes was performed on selective agar medium. At the initial screening stage 227 fungal strains from genera *Aspergillus* and *Penicillium* were tested. Colonies of 27 strains of fungi surrounded by visually detected zones with altered agar pigmentation were sorted out.

Aim of this work: to determine localization of glycerol-oxidizing enzymes synthesized by mycelial fungi and carry out electrophoretic analysis.

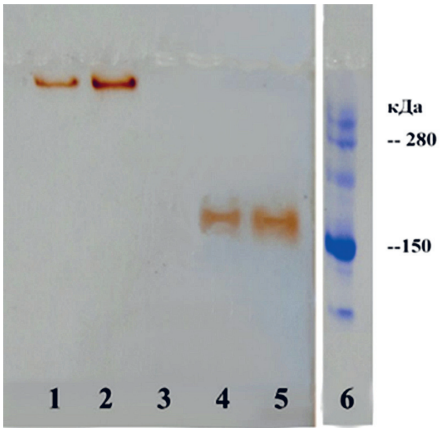

Fig. – Electrophoregram of fungal proteins catalyzing glycerol oxidation

Penicillium sp. 8 (1), A. fischeri MSK F-20394 (2), A. alliaceus HMM-IV-6 (3), P. fellutanum (4), A. carbonarius (5); control– glycerol-3-phosphate-oxidase «Sigma» (6)

Submerged cultivation of 27 selected strains was carried out. To promote localization of glycerol-oxidizing enzymes extracellular and intra-/cell-bound protein fractions were separated. It was found that the produced enzymes were intracellular or cell-bound. Based on the results of the electrophoretic analysis (fig.) 7 strains of mycelial fungi synthesizing glycerol-oxidizing enzymes were selected. It was established that 3 strains (*Penicillium sp*. 8, *A. fischeri* MSK F-20394, *A. varians* 3320) synthesized a protein with molecular weight of approximately ~ 400 kDa, and 4 strains (*P. fellutanum, A. carbonarius, A. niger* У3 ПК, *Penicillium sp.* 1000) – 160–165 kDa. According to the literature reports, glycerol-oxidizing enzymes produced by mycelial fungi may belong to glycerol oxidases (EC 1.1.3.B4, molecular weight ~ 400 kDa), glycerol dehydrogenases (EC 1.1.1.6, molecular weight ~ 160 kDa) and alcohol oxidases (EC 1.1.3.13, mol. weight ~ 160 kDa). Thus, 7 strains of mycelial fungi synthesizing glycerol-oxidizing enzymes were selected for further research.

BACTERIOSTATIC AND BACTERICIDE ACTIVITIES OF SOME 3D METAL COMPLEXES WITH 2-[2-(PROP-2-EN-1-YLCARBAMOTHIOYL)-HYDRAZINYLIDENE]PROPANOIC ACID

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Pyruvic acid thiosemicarbazones, their derivatives and 3d metal coordination compounds with these ligands manifest anticancer, antimicrobial, and antifungal properties. Therefore, the aim of this work is to determine the influence of introduction of the allyl moiety in the fourth position of the thiosemicarbazide fragment on biological properties of the corresponding thiosemicarbazone (H_2L) and biometal coordination compounds with this ligand.

Iron, cobalt, nickel, and copper salts react with pyruvic acid 4-allylthiosemicarbazone (H_2L) forming coordination compounds: Cu(HL)X (X=Cl^-, Br^-, NO_3^-); Cu(H_2O)(L); Cu(A)(L) (A = imidazole (Im); 3,4-dimethylpyridine (3,4-Lut)); $M(HL)_2X$ (M=Fe^{3+}, Co^{3+}; X=Cl^-, Br^-, NO_3^-) and $Ni(HL)_2$. The composition of these coordination compounds was determined using elemental analysis. Their structures were studied using magnetochemical research, IR spectroscopy, and X-ray diffraction analysis.

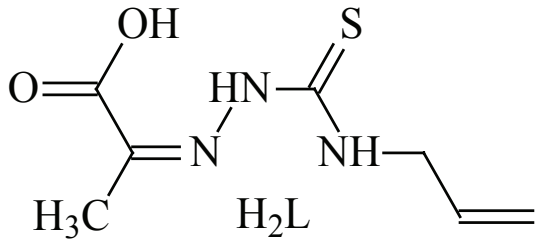

Fig. Pyruvic acid 4-allylthiosemicarbazone (H_2L)

The antimicrobial activity of these compounds was studied *in vitro* using the method of two-fold serial dilutions in liquid nutrient medium (meat infusion broth, pH = 7.0). The substances were dissolved in DMSO in such amounts that the solutions of concentration 10 mg / mL were obtained. The next dilutions were made using meat infusion broth. The antimicrobial activity was tested on standard strains of gram-positive (*Staphylococcus aureus* ATCC 25923), gram-negative *(Escherihia coli* ATCC 25922) bacteria and *Candida albicans.*

It was found that pyruvic acid 4-allylthiosemicarbazone (H_2L) shows neither bacteriostatic nor bactericide activities towards gram-positive and gram-negative microorganisms, but it inhibits the growth of *Candida albicans* at 500 μg / mL concentration. The coordination of the thiosemicarbazone H_2L to 3d metal ions changes the biological activity. The synthesized coordination compounds manifest antimicrobial and antifungal activities. The minimal inhibitory and bactericide concentrations are in the range of 31.25 - 1000 *μg / mL. The nature of the metal ion has the main influence on the antimicrobial activity of these coordination compounds. The antimicrobial activity significantly growth in case of copper(II) complexes. The introduction*

of amines in the inner sphere of copper coordination compounds also leads to the growth of inhibitory and bactericide activity. The nature of acid residue does not significantly affect the biological activity of the synthesized substances. Complexes Cu(HL)Br and Cu(Im)(L) show the highest antifungal activity.

The determinated properties of the synthesized biometal coordination compounds with pyruvic acid 4-allylthiosemicarbazone are of interest for medical practice for enhancement of the arsenal of antimicrobial and antifungal preparations.

This work was fulfilled with the financial support of the Project 18.80.07.17A/PS of the State Program.

THE INTESTINAL MICROORGANISMS OF SOME GENERA IN DIGESTIVE TRACT OF CHILDREN WITH VARIOUS HEALTH STATUSES

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Scientific research over the last 5 years has confirmed that the health of the digestive tract and of the whole body depends on the microorganisms that colonize it [1-3]. Intestinal microflora is thought to be the mirror of the health of the host organism [4-6].

That is why the main goal of this research was to study the intestinal micro-organisms of children with different health status.

According to the purpose, samples of intestinal (rectal) content from children of various ages and various health status of the digestive tract (sanogenic and pathological) were investigated. The genera *Lactobacillus*, as obligatory microorganisms, *Eschericha* - the optional ones, and *Proteus* - the putrefaction microorganisms, were considered.

The numerical value of the intestinal microorganisms of the nominated genera, determined during the investigative process (at 7, 90 and 300 days after birth) is shown in Tab.1. The results are expressed in decimal logarithms.

Table. 1 Quantitative index of microorganisms in children with various states of intestinal microbiocenosis

Lot	Type of microorganisms	Amount of microbial cells/1g of intestinal content, decimal logarithms (log), by age (days)			The difference between groups I and II (days),%		
		7	90	300	7	90	300
I	1 2 3	9,17±0,14 6,07±0,11 0	8,49±0,16 7,88±0,10 0	9,20±0,11 6,57±0,13 0			
II	1 2 3	7,43±0,14 8,74±0,11 5,26±0,17	6,84±0,17 9,77±0,12 4,17±0,07	7,11±0,10 8,07±0,16 1,17±0,07	-18,97 +43,98 +100,00	-19,43 +23,98 +100,00	-22,71 +22,33 +100,00

Note: Lot I is practically healthy (with the sanogenic state of intestinal microbiocenosis); II - sick children (with pathological condition).The genera of microorganisms: 1-Lactobacillus; 2-Escherichia; 3 Proteus.

The results confirm a considerable difference between the quantitative indices of intestinal microbial agents in healthy and sick children. This is observed in group II of children with pathological condition of the digestive tract, where the obligatory *Lactobacillus* genus is numerically in minority (by 18.97; 19.43 and 22.71% compared to the children of the group I respectively at the age of 7, 90 and 300 days after birth). The difference was even greater for optional bacteria and rotting bacteria. It is to be noted that the quantitative indices of the genus *Escherichia* increased respectively by 43.98; 23.98 and 22.33%. The last indication demonstrates that *Proteus* genus was present only in children of group II (sick).

Based on the obtained results, it can be stated that the microorganisms of the studied genera can serve as indicators of the state of health of the organism, because their quantitative indices respond promptly to the changes in children health status.

Conclusions

1. The sanogenic health state of digestive tube was characterized by: the numerical dominance of obligatory bacteria on the example of *Lactobacillus* genus; -minority of the facultative (*Escherichia)* and the lack of putrefaction (*Proteus*).
2. The pathological status of the digestive tract has shown inverse characteristics (the numerical decrease of the obligated microorganisms, the increased of facultative and the presence of putrefaction microflora).

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Yellow Biotechnology

Food Biotechnology

Nutrition Science

HIGH VIABILITY OF LACTIC ACID BACTERIA IN CULTURE-PROTECTIVE MEDIUM MODIFIED THROUGH MATHEMATICAL MODELING

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The milk-based medium contains optimal amount of substances which allows maintaining viability and preserving biotechnological properties of lactic acid bacteria cells. This proven fact determines the need of researches for optimization or modification of culture and protective media.

Experiments have been carried out to modify culture medium for lactic acid bacteria isolated from goat milk, which will allow the intensive biomass accumulation and the obtaining of a high viable count of microorganisms.

The growth dynamics, pH, titratable acidity and the number of viable microorganisms were monitored in cow milk –based medium and in the modified medium – goat milk based. Cultivation of strains was performed at optimal parameters in sterilized hydrolyzed cow and goat milk media for 24 h at 30±2 °C. The dilution method was used to estimate the number of viable lactic acid bacteria cells.

Studied strains, specially the strain *Lactococcus lactis* CNMN-LB-75, showed significant differences during cultivation on the control medium and the modified one, it has been established that the composition of the modified culture medium allows obtaining the highest productivity of autohtonous lactic acid bacteria isolated from goat milk. The highest accumulation of *L. lactis* CNMN-LB-75 biomass (1,4 mg / ml) was registered after 12 hours of incubation, at the value of pH = 4,5. The maximum count of cells was $3,8 \times 10^{10}$ at the stationary phase (Fig. 1). The growth parameters was determined by growth rate $\mu = 0,186$ oră$^{-1}$ and the shortest generation time g = 3,725 hours.

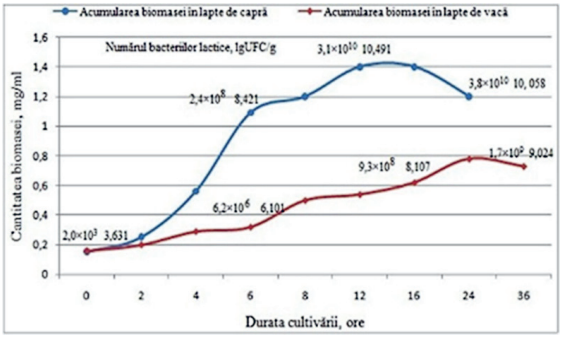


Fig. 1. Dynamics of *L. lactis* CNMN-LB-75 strain development and biomass accumulation during the cultivation on cow milk –based and goat milk-based media.

The protective medium plays an important role in maintaining of the viability of microorganisms during freeze-drying. The base of protective medium for preserving lactic acid bacteria was optimized by substitution of cow milk to goat milk. The mechanism for optimizing protec-

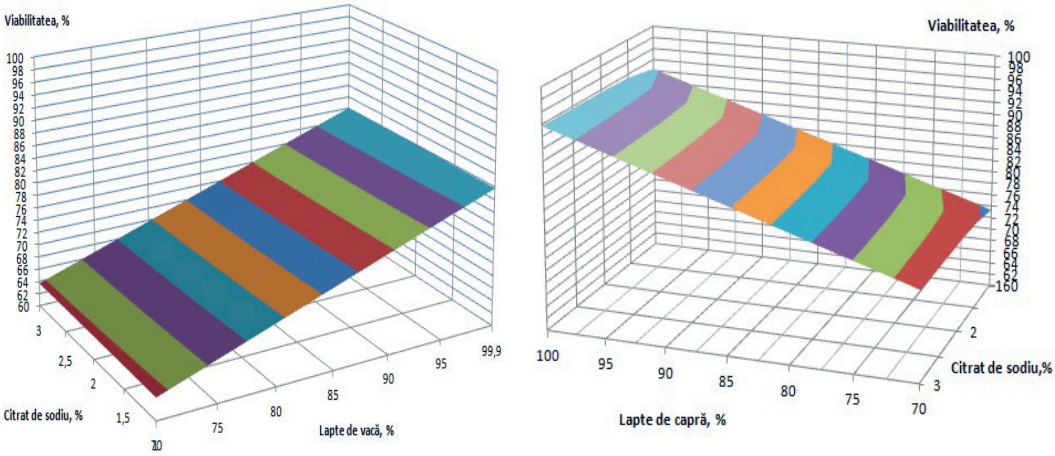
tion medium was established by developing mathematical model for calculating the viability index of bacteria considering all possible interactions between milk and phosphate buffer solution (sodium citrate) with the addition of protective substances. The impact of protective cow milk-based and goat milk-based media on strains viability were studied after freeze-drying.

In the result of processing the experimental data, was obtained the regression equation which describes in natural values ($p < 0.05$) the changes of lactic acid bacteria strains viability depending on the content of base protective components in the protective medium:

$$Y=-194,12+6,60\times L+0,40\times C-0,04\times L^2-0,05C^2$$

where Y is viability of LAB (%), L is goat milk content (%),C is sodium citrate content (%).

A typical 3D surface plot, showing the compared effect of cow milk with buffer solution and goat milk with buffer solution is presented in Fig. 2.



a) b)

Fig. 2. 3D surface plot displaying the influence on viability of freeze-dried strains of lactic acid bacteria of: A) sodium citrate and cow milk, B) sodium citrate and goat milk

This study demonstrated that mathematical model was useful in evaluating the effects of factors leading to a higher survival of the lactic acid bacteria cells.

The analysis of obtained results shows that the use of goat milk resulted in increased viability of lactic acid bacteria strains during freeze-drying, in comparison with cow milk-based protective medium, that is important for maintaining their biotechnological properties.

ELUCIDATION OF THE BIOR REMEDY IMPACT ON THE FEMALE RABBITS` LACTATION CAPACITY IN AN IMPLEMENTATION STUDY

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Animal breeding and exploitation in the conditions of intensification and industrialization of this branch of economy represents a difficult chapter of the modern animal husbandry. In relation to the type and degree of stress factors aggression, unconditionally occurring in such circumstances, the livestock sector is experiencing significant disturbances in both the health and animal welfare, as well as in the productive and reproductive potential of animals.

The progress within the basic sciences have allowed better counteraction of these phenomena, giving priority to biologically active remedies of natural, and especially plant origin. The in-depth study of the animal physiological-metabolic processes, treated with biologically active remedies, revealed some of their mechanisms of action, in particular of the BioR remedy, obtained by modern biotechnologies, from *Spirulina platensis* cyanobacterium.

The purpose of the research was to evaluate the influence of the BioR remedy on the health and reproductive potential of the female rabbits, in an implementation study. The researches were conducted under physiological conditions of production, on the New Zealand breed, during the reproductive cycle. The study included a group of 60 female rabbits, divided into 2 lots, 30 animals each. The control lot of rabbits were injected intramuscularly with physiological solution of 1,0 ml/head, twice: 5-7 days previous the mating, and the second time, on the 14th day of gestation. The tested remedy (BioR) was administered in the same terms to the animals from the experimental group, at the dose previously elaborated through two scientific and practical studies – 1,5 ml/head. The female rabbits, as well as their offspring were permanently monitored. During the research, the body temperature, the number of respiratory movements per minute and the cardiac frequency were determined, at 7 female rabbits, in both groups. For laboratory investigations, at the beginning of the study, blood was collected from 5 animals, randomized, and the second time, from 5 animals from each group, at the end of the study, at the 45th day of lactation. The offspring were weighted all at once (from each female rabbit): at the 1st day of birth, and afterwards, at the 10th, 21st, 30th day after birth. The 5th weighing of the bunnies was performed individually, at the end of the study, at the 45th day after birth.

The BioR remedy, during the 80-day experiment, did not cause side effects or other deviations in the health of the female rabbits and their offspring. It has also been established that the investigated clinical and hematological parameters had positive tendencies of manifestation, reflecting both the harmlessness of the remedy, as well as its adaptive and antistress action. The study shows that BioR determines the strengthening of the reproductive potential in female rabbits, which can be defined by: increase of the prolificity, of the live rabbit's number at birth, as well as the body weight of a rabbit at birth. The lactation capacity, which was determined on the 21st day of lactation, was higher by 941,0 g or 39,0% in relation to the control group, reflected in better productive parameters, at the end of the study.

In conclusion, we assert that both the results of the evaluation of the reproduction parameters changes in the BioR treated rabbits and the productive parameters in their descendants, over a period of 45 days of breeding, show that the tested remedy has a good impact on the investigated parameters.

THE INFLUENCE OF BIOR AND BUTOFAN REMEDIES ON THE HEALTH AND PRODUCTIVITY OF ADULT QUAILS UNDER RECONDITIONING

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Nowadays, it is well known that animals bred under farm conditions are subject to technological stress. Therefore, the productivity and quality of the products obtained from such animals decrease. A decrease in the productivity and quality also takes place using a wide range of drugs, many of which are unsafe for animals and humans. A number of studies suggest that, from a wide range of natural products that exhibit therapeutic and productive effects, a larger perspective present those obtained from microalgae. Therefore, a huge theoretical and practical interest presents the study of the mechanisms of action of biologically active substances of natural origin, in particular of the BioR remedy, obtained by modern technologies from *Spirulina platensis*, on the clinical and hematological status, as well as on the productivity of adult quails placed under reconditioning.– Butofan, is of.

The research of the biological activity of the above mentioned remedy was carried out in a comparative study with Butofan and physiological serum (sol. 0,9% NaCl), on a group of 150 adult quails, at the end of the laying cycle, put under reconditioning, belonging to the English White breed, weighting 296,4-301,0 g, and divided into 3 lots, 50 birds each. All three lots of quails were set up randomly, while respecting the principle of analogy. All the mentioned remedies were administered intramuscularly, in the same place - the pectoral muscles. For hematological investigations, at the onset of the study, 5 blood samples were collected, and during the study (at the 28th day), as well as at the end of the experiment (at the 50th day), 5 blood samples were taken from each lot. The quails were weighed individually, at different stages of the study (at 10-14 days), and on a daily basis, the eggs were collected and counted.

The BioR and Butofan remedies, during the 50-day study, did not cause any adverse effects or other deviations on quail's health. It has been established that both the clinical and hematological parameters investigated manifested positive tendencies. Thus, the tested remedies exhibited adaptive and antistress properties, reflected also in lower body temperature at the end of the study: in the lot treated with BioR, by 0,32°C, and in the lot treated with Butofan, respectively by 0,18°C in relation to the reference lot. These are considered to be good results for both remedies, and in particular for the BioR remedy.

Similar results regarding quail body temperature and respiratory movements have been reported. Studies have shown that the hematological parameters investigated: RBC, HB, HCT, MCV, MCH and MCHC, both for intact quails and the experimental groups, do not show major deviations throughout the study. In addition, the obtained results highlight the fact that the tested remedies show good results regarding the values of the investigated parameter in this category of birds. A special place in testing drug remedies on animals also has the study of the productive parameters. In our study, the evolution of the body weight in quails had a negative dynamic. The body weight loss in birds from the reference group was of 16,46 g, and in the groups treated with BioR and Butofan of 5,2 g, and 8,76 g, respectively. The laying qualities of the quails treated with the remedies taken in the study were superior to those in the reference group.

In conclusion, we underline the fact that the tested remedies, especially BioR, show positive health and productive effects on adult quails put under reconditioning.

EFFECT OF CYANOBACTERIAL SUBSTANCES ON THE AMINO ACID COMPOSITION OF *STREPTOMYCES MASSASPOREUS* CNMN-AC-06 BIOMASS, CULTIVATED ON COMPLEX MEDIUM R

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Amino acids are the most important regulators of microbial metabolism. The compounds of this group are the most important elements of the normal nutrition of humans and animals. An important element in the intensification of industrial husbandry is the creation of balanced feeds of all necessary components, especially of amino acids. An alternative way of balancing the feed by the content and composition of the protein is the use of feed additives.

The aim of our research was to study the amino acid composition of the *Stremptomyces massasporeus* CNMN-Ac-06 obtained by cultivation on a complex medium R with the addition of cyanobacterial agents.

The object of the study was the strain *Streptomyces massasporeus* CNMN-Ac-06, isolated from soil of the central part of Moldova and stored in the National Collection of Non-Pathogenic Microorganisms of the Institute of Microbiology and Biotechnology (Republic of Moldova). Cyanobacterial preparations - BioR and polysaccharides (Psh*ZnS) were used in various concentrations, to determine their influence on the amino acid composition of actinobacterial biomass. The amino acid composition of the obtained biomass was determined by ion exchange chromatography on an amino acid analyzer AAA-339 M "Microtehna" (Czech Republic).

The comparative analysis of the amino acid content in the biomass of the *Streptomyces massasporeus* CNMN-Ac-06 showed a pronounced increase in the number of individual amino acids in the case of cultivation on the medium with extracts of cyanobacteria. For example, in the biomass obtained on the nutrient medium with 10.0 % BioR, the quantity of threonine increased by 29.79 %, leucine by 38.22 %, arginine by 61.82 %, histidine by 66.49%, methionine by 121.39 %, cysteine by 98.89 %, cysteic acid - by 483.20 % compared to the control. When polysaccharides were added to the nutrient medium, the increase in the content of the following amino acids was observed: methionine by 54.9 %, threonine by 42.82 %, proline by 63.1 %, alanine by 43.6 %, valine by 55.3 %, histidine by 74.8 %, ornithine by 216.3 %.

Our experiments demonstrated the ability of the strain *Streptomyces massasporeus* CNMN-Ac-06 to accumulate in biomass, in different amounts, 21 amino acids, including 9 essential. The tendency of a significant increase in the amount was mainly pronounced for the essential amino acids.

Thus, the obtained data allow us to consider the use of cyanobacterial extracts to increase the biosynthetic activity of *Streptomyces massasporeus* CNMN-Ac-06, in particular, to increase biomass accumulation with improved qualitative and quantitative composition of amino acids.

POSSIBILITIES OF USING SOY PROTEINE ISOLATE FOR THE PACKAGING OF *JUGLANS REGIA* L. NUTS

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The walnut has gained popularity because of its specific attractive organoleptic properties and high levels of essential fatty acids and bioactive components such as antioxidants. Walnut production is widely distributed all over the world and currently it ranks third in terms of global nut production after cashews and almonds (Pereira et al., 2008).

Analysis of tendencies in consumer packaging shows increased use of synthetic polymeric materials [Kenneth Marsh Ph.D. et al., 2007]. Nevertheless, the rapid development and implementation of them greatly exacerbates environmental problems in the developed countries of the world. The degradation of traditional polymeric materials takes a long time (tens and hundreds years).A promising solution for the problem of environmental pollution with polymeric waste is the development of a wide range of natural raw materials [Дубинина А. А. 2010]. The switch to new conservation methods will allow manufacturers to focus their efforts on improving productivity and quality, so as to expand the sales market and cover a wider area with regard to export directions. [Jung H. Han 2005].

For the research were used high quality Greek nuts (*Juglans regia* L.) of the "Cogalniceanu" variety, the 2016 harvest. Food granules were obtained on the basis of soy protein isolate (L.Atarésa, C. De Jesúsa 2010). The analysis of the obtained food packaging film solutions was accompanied by the determination of quality indicators by organoleptic evaluation of the samples immediately after the coating process and during the storage period. The viscosity of the solution with IPS concentrations was determined: 3.75, 3.5, 3.0 g. It has been observed that the higher solution viscosity provide the more uniform film and the better adhesion. The surface of the film was analyzed using the Dino-Lite Basic AM200 microscope with the application of different fonts. It has been observed that the properties of the samples evolve depending on the concentration of the isolate of soy protein. The results show that samples with 3.5 g of soy protein isolate have an excessive roughness with high inclusions.

In conclusion, the utilization of soy protein isolate in packaging film solutions for the subsequent preservation of crushed nuts and quarters is an effective way to keep the quality of the products under control.

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THE IMPACT OF ORGANIC FOOD ON THE MOLDAVAN MARKET

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According to the latest data from the Organic Agency, the market of food products from organic farming has again experienced a strong increase in 2011. Indeed, it rose from 3.385 billion to 3.755 billion EUR, an increase of + 11% (10.8% in 2010). Consumption of organic products almost doubled compared to 2007 (€ 2 billion) and the organic food market reached 2.4% of the total food market (compared to 1.3% in 2007) [3, 5]. The market share of organic products is more or less important depending on the sector. For example, it exceeded 10% for milk and eggs and reached almost 6% for the 14 fruits and vegetables most consumed in Europe [1, 2, 4].

The research problem underlying this study is to collect information on market trends and needs of the food market in Moldova and in particular on attitudes towards these products, information to support the future research initiative. The study was conducted on a group of 225, 71.56% - from Chisinau. The questionnaire consisting of 14 questions and face-to-face interview were used as research methods. The questions were focused on drinking and food behavior, as well as on the perception of the difference between the concepts of eco, bio, natural and biological.

Organic farming is now entering the agricultural world, where everyone is questioning how to make production systems and development models more sustainable. This seems very promising for us. The research will now take a new dimension and three main directions:

- Evaluation and improvement of techniques that meet the specifications of the biological agriculture
- The study of the functioning of production systems, market studies of consumers and producers, to target their problems and their expectations.
- The stimulation of organic consumption through good communication to the consumer (highlighting the qualities of the products) and to the citizen (explanation of the ethical and environmental foundations of organic farming). For this reason, each year, the organic sector is mobilized for the National Information and Promotion Week for Organic Agriculture, coordinated at the national level by the Bio Agency.

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THE INFLUENCE OF SUBTITUTING NACL WITH KCL ON TELEMEA CHEESE: PHYSICO – CHEMICAL COMPOSITION, TEXTURAL AND SENSORIAL PROPERTIES

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Sodium chloride is an indispensable constituent of white brined cheeses. It is involved in flavor and hardness improving, water activity and bitterness reducing, controls the enzymatic activity and bacterial growth and contributes to cheese preservation. Several studies have shown the negative impact of high sodium chloride content from processed cheeses on the consumers health. The most common additive used to decrease the levels of sodium chloride without affecting the cheese quality is potassium chloride.

The aim of this study was to examine the effect of total or partial substitution of sodium chloride by potassium chloride on the physico – chemical, textural and sensorial characteristics of Telemea cheese samples during ripening at 4°C for 28 days.

Telemea cheese was manufactured by using fresh sheep milk with 7% fat. Rectangular blocks (≈ 150g) of pressed curd were placed into plastic containers using 4 brine solutions (20%, wt/wt) with different concentrations made from different NaCl/KCl combinations as follows: (NaCl (A), KCl (B), 1NaCl:1KCl (C) and 1NaCl:2KCl(D)).

The changes of salt contents for the experimental cheese samples are summarized in Fig. 1. The value of this parameter at the beginning of the ripening period was 2.75 ± 0.23% and reached by the end of this period a maximum value of 5.40 ± 0.19%. The total substitution of NaCl with KCl increased the salt content significantly by 50.92%, and reached a higher concentration for cheese sample B. This results are in agreement with those reported by Katsiari *et al.* (1997) in the case of partial substitution of NaCl by KCl in Feta cheese and Bakirci *et al.* (2011) in the case of Turkish white pickled cheese.

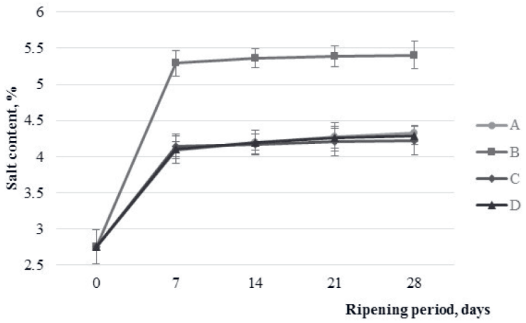

Figure 1. Evolution of salt content in Telemea cheeses during 28 days of ripening

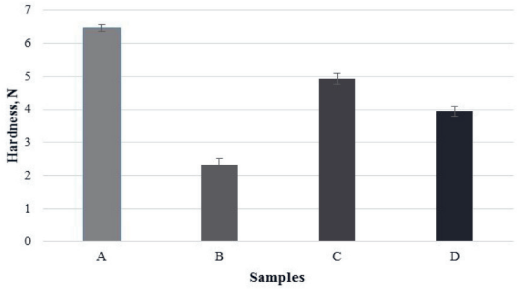

Figure 2. TPA hardness for Telemea cheese

The results of hardness of the all experimental cheese samples are presented in Fig. 2. The highest values for hardness were registered for cheese samples ripened in brine solution

A (6.47 ± 0.11 N) and the lowest for the samples ripened in brine solution B (2.32 ± 0.19 N). The total substitution of NaCl with KCl influenced this textural parameter. Similar results were reported by Kamleh *et al.* (2012) for Halloumi cheese.

The sensory analysis, using a consumer acceptability test, of the experimental Telemea cheeses showed that cheeses salted with NaCl/KCl mixtures or with KCl were found acceptable by the consumers. These results are in agreement with Ayyash *et al.* (2012), and Katsiari *et al.* (1997; 1998) in their studies on the effect of KCl or NaCl/KCl mixtures on Akawi, Feta, and Kefalograviera cheeses.

This study confirms that potassium chloride is a viable alternative to total or partial replacement of the sodium chloride in Telemea cheese.

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PUMPKIN PASTE AS A NATURAL AGENT FOR PASTRY TEXTURE

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In the Republic of Moldova pumpkin has good growth conditions, being cultivated in considerable proportions and usually used without any industrial pre-processing. Due to a wide variety of healthy nutrients, pumpkins have anti-inflammatory, anti-oxidant, anti-cancer and anti-diabetic properties. Nowadays pumpkin is used as a supplement (natural food coloring, vitamins, minerals) powder or paste, in bakery technologies, pastry, lactic acid products, canning industry etc. in order to improve the nutritional value of the final product. [T. Schiopu, 2012]

The purpose of this research was to elaborate a technology of manufacturing pastry products using the pumpkin paste to diversify the assortment of functional vegan products and to improve the nutritional value of these products thanks to the pumpkin's rich variety of vitamins, micro and macro-nutrients.

In our study, pumpkin paste has been added in a quantity between 15% and 40% from the mass of the wheat flour, used for the preparation of cakes made with plant-based margarine and cakes made with sunflower oil, buns of dough, vegan sweet bread and cookies made with margarine and plant-based mayonnaise.

The quality of obtained samples was appreciated by 5 point sensory evaluation scheme and the profile diagram according to GOST.[ISO 6658: 2005. Sensory analysis.]. Thus, the influence of the addition of the pumpkin paste on the volume and the shape, the appearance, the crust color, the porosity, the core consistency, the smell and the taste of the final products, comparing to the blank samples, was studied.

Analyzing the results achieved at the evaluation of the organoleptic indices of buns made out of vegan sweet bread dough, we found the highest values for all of the organoleptic indices at the addition of 20% of the pumpkin paste. The cakes made with plant-based margarine and sunflower oil also showed better results for the cakes with a 20% pumpkin paste addition, with the highest organoleptic indices – volume and shape, crust aspect, core color and consistency, smell and taste. [ГОСТ 15052-2014.]. At the evaluation of the cookies made with margarine and plant-based mayonnaise, we noticed the same effect of 20% pumpkin paste addition on the as taste, color, tenderness, which reflects its advantage over the other products. [ГОСТ 24901–2014.]

The study showed that adding 20% of pumpkin paste to the recipe of vegan pastry products improves, not only the nutritional values of the products, but also their sensory indices such as the appearance, taste and color.

In conclusion, we can propose the improving of pastry products quality by adding pumpkin paste and substituting or excluding some basic and auxiliary raw materials from the classic recipes (the egg).

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OHMICALLY HEATED COURGETTES (*CUCURBITA* PEPO *VAR. OBLOGA*) PUREES ESPECIALLY DESIGNED FOR DYSPHAGIA

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Dysphagia is a dysfunction, which causes the impossibility of food or liquid movement from mouth to stomach. Usually the causes of dysphagia are muscles or nerves problems, which can be painful and can be supported by people of every age. Even so, more exposed are children and elderly people. In dysphagia, besides treatment, an important factor represents the food, which must be in accordance with the necessities. Various types of purees compose the meals of people with dysphagia.

The aim of this study is to design and characterize some courgette puree with sodium alginate addition, preserved by ohmic heating. For this study, 3 types of courgette purees with 1, 2 and 3% sodium alginate addition and a control sample ohmically heated at 20 V/cm for 3 minutes were chosen. The courgette purees were manufactured from fresh peeled and washed courgettes, mashed at 1900 rot/min for 3 minutes.

The ohmic heating process used as preservation method has some advantages as better product quality, less cooking time, lower capital cost, better energy efficiency (Darvishi, et al., 2015) and an environment friendly processing. All courgette purees exhibited a non-Newtonian behavior (Alvarez and Canet, 2013). The highest values of viscosity are dependent on the sodium alginate concentration. The Ostwald Waele model is capable of simulating the rheological properties of all the samples with a very good fit. Purees texture is influenced by the sodium alginate addition, which is expected, while the aim of hydrocolloids using is to assure more softness and palatability. Cohesiveness is a textural parameter with important implication in the design of food for people with dysphagia, because it describes the degree in which the aspiration in airways is prevented. The cohesiveness values ranged from 0.52±0.01 to 0.67±0.01 increasing with the sodium alginate concentration.

Based on these results and considering the other publishers studies, ohmic heating can be successfully used to provide shelf stable products.

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THE QUANTITATIVE LEVEL OF SOME INTESTINAL MICROORGANISMS DEPENDING ON THE FOOD FACTOR

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It is now recognized that in the process of maintaining the optimal function of the digestive system an essential role is fulfilled by the food factor (for example fermented milk products). In this direction, major attention must be paid to additives and newly developed food rations, because of their role in the process of maintaining or modifying the functionality of the nominated system. The reported ones are confirmed by their influence on intestinal microflora which fulfills a multifunctional role in digestion and immunity. Other ones are recognized as pillars of health status coordination to maintain or develop pathology.

The laboratory research was carried out in two sets of experiments. In the first, we used laboratory animals (guinea pigs) divided into 4 equal lots (5 each) and three food additives (PRESANE, STEM and MEDULAC - WM). The second experimental series was done in the same laboratory conditions, on rats of the Vistar line, with four variants of food rations balanced, according to basic components (proteins: lipids: carbohydrates): I. 14%: 28%: 58%; II. 16%: 26%: 58%; III. 18%: 25%: 57%; IV. 20%: 23%: 57%.

In the course of the investigation, quantitative indices of intestinal microorganisms of some genera binding to the digestive tract (*Bifidobacterium* and *Lactobacillus*) were studied. As a result of the first series of experiments, compared to the initial, it was found that all the food additives tested helped to increase the quantitative indications of bifidobacteria and lactobacilli, being in animals from experimental lots (II-IV) compared to the original, respectively, by 28.62 % and 31.35%; 42.54% and 36.32%; 5.26% and 19.07%. The data differences were impressive, compared to those in the control group. Thus, it can be stated that all the results obtained in this series confirmed the dependence of the numerical value of the intestinal microorganisms of genera *Bifidobacterium* and *Lactobacillus* on the food factor.

Analyzing the second series results, we can report that the tested food rations have been beneficial for bacteria of the binding genes, but in a differentiated way. It was found that the most optimal action can be considered as variant II, because of increase of the numerical value of the obligatory representatives of the intestinal microflora of the genera *Bifidobacterium* and *Lactobacillus* (respectively by 25.14% and 18.13%), compared to the original. At the same time, it was found that the difference between the results obtained in the variant II was even larger compared to the control group and was higher for bifidobacteria and lactobacilli by 27,10% and 19,03% respectively.

Therefore, based on the results of the present scientific researches of two series of experiments it has been shown that the quantitative level of the intestinal microorganisms of the digestive tract (genera *Bifidobacterium* and *Lactobacillus*) can serve as a criterion of appreciation of the degree of action of the food factor on the digestive tract microbial disease status (sanogenic or pathological). Such a criterion is of scientific interest and can be recommended for practical application in both the preparation of new additives and food rations composition. This is confirmed by the fact that the intestinal microorganisms of the nominated genera

have responded promptly to the tested food factor by modifying their quantitative level, because he always found himself depending on him.

This is why we consider that obtained experimental data argue the opportunity to recommend selected variants of food additives and rations for their use in order to maintain optimal or sanogenic microbial digestion in the digestive tract.

Blue Biotechnology

Aquaculture

Costal and Marine Biotechnology

THE ACTION OF ZN(II) ACETATE ON ADAPTIVE CAPACITY OF *SPIRULINA* IN RESPONSE TO CHANGES IN THE LIGHT REGIME

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Intensive cultivation technologies of microalgae and cyanobacteria imply the presence of chemical stimulators in mineral medium that orient the activity of microorganisms in the direction of super synthesis of some biologically active compounds. The presence of chemical stimulators may be a factor leading to the loss of adaptive capacity of cyanobacterium to changing cultivation conditions with accumulation of oxidative degradation products of lipids.

In order to monitor the adaptive capacity of spirulina, grown in the presence of zinc acetate as stimulator of protein synthesis, cyanobacterium has been subjected to light-induced oxidative stress. During the adaptation period, *Spirulina platensis* was grown in the presence of 15 mg/l of Zn(II) acetate under optimal conditions with continuous illumination. On day 3 of cultivation cycle, corresponding to early exponential growth phase, spirulina was exposed to stress by reducing light period up to 4 hours from 24 hours. Thus, lighting regime was with photoperiodism of 4 hours light/20 hours dark. This regime was followed until day 7 of cultivation. It was used as control sample spirulina, grown in the absence and presence of zinc acetate under optimal conditions. In spirulina biomass, collected every 24 hours, was determined the content of malondialdehyde (TBARS assay).

Under optimal conditions, in variants with supplementing the cultivation medium with zinc acetate, TBARS test values are lower than values determined in control during exponential phase. Reducing light period during exponential growth phase of spirulina in the lack of zinc acetate did not change the content of malondialdehyde in biomass. Therefore, the reduction of light period during exponential phase of spirulina was not a stressful factor for culture.

In spirulina culture, grown in the presence of zinc acetate and exposed to light stress, it was determined an increase of malondialdehyde content. Similar values with control sample of malondialdehyde content in spirulina biomass were determined on day 4 of cultivation cycle. Therefore, reducing the light period to 4 hours in the first 24 hours of illumination stress did not alter biosynthetic activity of spirulina in the presence of chemical stimulator. In the next day of exponential phase, TBARS test values have increased with 38% from the previous day, which was with 92% more compared to malondialdehyde content in control biomass. At the end of exponential growth phase, malondialdehyde content increased with overall 29%. The beginning of stationary phase was marked by decreasing with 15% of malondialdehyde content.

Therefore, spirulina culture, grown under conditions of stimulating biosynthetic activity on the basis of zinc acetate action, became more vulnerable and reducing the light period was a significant stress factor for cyanobacterium.

THE USE OF CHEMICAL COMPOUND $Fe_3Se_3O_9 \cdot 6H_2O$ TO OBTAIN SPIRULINA BIOMASS ENRICHED WITH SELENIUM AND IRON

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Intensive researches are currently directed toward the study of microalgae and cianobacteria as a potential source of bioactive substances (proteins, vitamins, essential amino acids, β-carotene, etc.) and for their use as valuable food additives and medicines. One of the most promising among them is the cyanobacterium *Spirulina platensis*. The quality of its biomass can be improved by directed synthesis, changing the composition of the culture medium and using of new chemical compounds. The use of selenium for his purpose is of particular interest.

Few scientific papers report data on the effect of only three compounds containing selenium (Na_2SeO_3, Na_2SeO_4 and H_2SeO_3) on the cyanobacterium *Spirulina platensis*. Their effects refer to the improvement of spirulina biochemical composition: increase of the protein content, especially the amount of phycobiliproteins; of the content of polysaccharides, depending on the fraction; and also selenium enrichment of biomass. Selenium is an essential trace element for the normal development of living organisms. It activates the processes of tissue respiration, regulates oxidation-reduction reactions, and affects immunological activity, protein metabolism, especially the metabolism of sulfur-containing amino acids. Its therapeutic (antiviral, antimicrobial, anti-inflammatory etc.) characteristics are also widely known.

The use selenite of iron $Fe_3Se_3O_9 \cdot 6H_2O$ during spirulina cultivation allows the accumulation, along with selenium, of another important element - the iron. The most important function of iron in the body is the production of red blood cells. A large number of enzymes require iron as cofactor for their functions. This element is involved in maintaining the normal state of the immune system, supporting the formation and maturation of T-lymphocytes. Deficiency of iron has a negative effect on mental development.

In our studies, spirulina cultivation was carried out for 144 hours, in Erlenmeyer flasks with 100 ml Zarruk nutrient medium, with a specific ratio of macro- and microelements for normal development and growth. The necessary conditions for the normal biosynthesis of all intracellular components of *S. platensis* were observed: temperature of $28^0 \pm 1^0$C, illumination of 30003 lux, pH = 9,5-10,0. The chemical compound $Fe_3Se_3O_9 \cdot 6H_2O$ was introduced on the first day of cultivation in concentrations of 10, 20 and 30 mg/l.

According to the obtained data, selenite of iron in all of investigated concentrations has a positive effect on the productivity of cyanobacterium *S. platensis* and enriches its biomass with selenium and iron. The best option was noted when this compound was added at a concentration of 30 mg/l. The productivity was 10% higher than the control sample, and the accumulation of selenium and iron was 280 mg% and 318 mg%, respectively. Thus, the $Fe_3Se_3O_9 \cdot 6H_2O$ compound can be used to produce biomass of cyanobacterium enriched with selenium and iron, that can served as a source for production of preparations with antitumor, antiviral, antibacterial and antianemic properties.

MODIFICATION OF IRON REDUCING POWER IN *SPIRULINA* BIOMASS IN RESPONSE TO INDUCED STRESS

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Oxidative stress-induced conditions mobilize cell reserves in order to mitigate the harmful effects of excessive free radicals production and to stop the processes of lipid peroxidation. The ability of cells to decrease the intensity of lipid peroxidation reactions can be assessed by determining the iron reducing power.

In order to monitor the adaptive capacity of spirulina, grown in the presence of Zn(II) acetate and Fe(III)-alanine as stimulators of protein synthesis, cyanobacterium has been subjected to oxidative stress induced by fluctuating light regime. Throughout the adaptation period, *Spirulina platensis* was grown in the presence of 15 mg/l Zn(II) acetate and 50 mg/l Fe(III) alaninate under optimal conditions with continuous illumination. On day 3 of cultivation cycle, corresponding to the early exponential growth phase, the culture has been exposed to light-induced stress by reducing light period within 4 hours of 24 hours. This regime has been followed until day 7 of cultivation cycle. It was used as control spirulina, grown in the absence or presence of chemical stimulators under optimal conditions. In algal biomass, collected at intervals of 24 hours, it was determined the iron reducing power, expressed as mg/ml ascorbic acid equivalents.

Under conditions of continuous illumination, tested compounds increased significantly the capacity of biomass to reduce iron ions. In experimental variants applying Zn(II) acetate, the value of iron reducing power in early exponential growth phase was 36% higher compared to control samples. Elevated levels were maintained throughout cultivation cycle and in early stationary phase were 30-39% higher. Fe(III) alaninate from cultivation medium led to an increase of iron reducing power of 1.4 times at the beginning of exponential growth. On day 5 of cultivation, iron reducing power was about 65% higher compared to control. In stationary phase, iron reducing power was about 87% higher compared with control samples.

Under conditions of light-induced stress reducing to 4 hours of light period, the presence of chemical stimulators in spirulina cultivation medium decreased the values of iron reducing power.

Zn(II) acetate decreased iron reducing power with 62% on day 5 of spirulina cultivation under light-induced stress, culture being in exponential growth phase. In the following days, iron reducing power of biomass was maintained at values below control samples.

In the presence of Fe(III) alaninate, on day 5, iron reducing power decreased by 53% and remained low during the exponential phase.

Reducing this parameter under light-induced stress revealed the involvement of supplemented compounds in biosynthetic processes of spirulina that depleted the antioxidant capacity of culture. Therefore, the iron reducing power assay in spirulina biomass can confirm induced oxidative stress.

APPLICATION OF GOLD AND SILVER NANOPARTICLES IN CULTIVATION TECHNOLOGIES OF MICROALGAE *DUNALIELLA SALINA*

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Nanotechnologies are in continuous development with fields of inexhaustible application, such as the production of building materials and dyes, food technologies, pharmaceutical and cosmetics industry, environmental protection and medicine.

In parallel with the development of nanomaterials manufacturing technologies and diversification of their use areas, researches on the issue of their toxicity and environmental impact become very current. However, some studies have revealed positive effects of these materials on cell growth or metabolic activity.

Results of nanoparticles action on microalgae are very varied, and their effects will depend on nature and size of nanoparticles, but also tested microalgae species and cultivation conditions.

In order to elucidate the action of very small gold/silver nanoparticles on halophile microalgae, experiments were carried out to cultivate green alga *Dunaliella salina,* in the presence of gold and silver nanoparticles. There have been used Au (PEG) and Ag(PEG) nanoparticles with the size of 5 nm in polyethylene glycol. Nanoparticles were supplemented to Ben-Amotz mineral medium with salinity of 120 g/l from the first day of cultivation.

Concentrations of 0.054-0.108 mg/l AgNP had stimulatory effect. The content of biomass was 12-33% higher compared to control. A possible moderate toxic effect was set in the experimental variant with applying nanoparticles in concentration of 0.135 mg/l, which reduced biomass content with 15%. Doubling the concentration of nanoparticles to 0.27 mg/l has not altered biomass content of microalgal culture.

It was investigate the impact of nanoparticles on biosynthetic activity of microalga. Thus, concentration of 0.054 mg/l AgNP enhanced the content of β-carotene in algal biomass with 33%. An increase of 20% of carotene content in biomass was also determined in variants with low concentrations of AgNP in cultivation medium. Therefore, no dependence between AgNP concentration in cultivation medium and microalgal culture response was established. The tested concentration of silver nanoparticles is not toxic for *Dunaliella salina*. It was established a concentration with stimulating effect on biomass production and β-carotene synthesis.

In case of applying gold nanoparticles a stimulating effect has been revealed. Maximum accumulation of dunaliella biomass was determined by adding the concentration of 0.081 mg/l AuNP, for which biomass production has increased by 21%. Concentration of 0.27 mg/l AgNP, which was the maximum of applied doses, did not altered biomass accumulation, but resulted in a 20% increase of β-carotene content in biomass. The tested concentration of gold nanoparticles are not toxic for *Dunaliella salina*..

In conclusion, we can state that Ag (PEG) and Au(PEG) nanoparticles with 5 nm size, supplemented to growing medium, have no toxic effects and can be applied in cultivation technologies of microalgae *Dunaliella salina* as stimulants of carotenogenesis.

INDUCED OXIDATIVE STRESS - A BIOTECHNOLOGICAL TOOL IN PHYCOBIOTECHNOLOGY

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Phycological production is becoming more attractive due to several advantages, one of which is the use of very compact surfaces for the cultivation process. Moreover, phycological biomass is a profitable source of proteins, polysaccharides, lipids, pigments and other products with high biological activity. These benefits determine the obvious interest of biotechnologists for mass cultivation of microalgae and cyanobacteria in different type bioreactors. Cultivation under industrial conditions are associated with oxidative stress, and this can compromise biomass quality. For these reasons, the stress associated with the accumulation of free radicals and degradation products of macromolecules, is regarded as a major challenge, an economic risk factor, as well as a risk to human health. However, a moderate stress may be associated with certain benefits for industrial cultures of microalgae and cyanobacteria. It would allow increasing biomass production, a more rapid accumulation of polysaccharides, altering the contents of pigments, etc.

In order to enhance the performance of an industrial strain, in this case *Spirulina platensis* CNMN-CB-11, we showed how to apply a stress condition as an efficient tool of biotechnology. *Spirulina platensis* CNMN-CB-11 was adapted to grow under continuous illumination. It was shown that the application of photoperiodism (12 hours of light and 12 hours of darkness) can induce a state of oxidative stress in culture, as indicated by a considerable increase in the amount of malondialdehyde by 0.5-3.7 times. At the same time, light stress induced an increased accumulation of phycobiliproteins (up to 45% higher than under conditions of continuous illumination), carbohydrates (2 times higher) and lipids.

Another stress factor for spirulina is temperature. During hyperthermia (cultivation of spirulina at 40°C), on the background of low amounts of the main biologically active components, in spirulina biomass increased significantly the activity of primary antioxidant enzymes - superoxide dismutase, catalase and peroxidases. Conditions of hypothermia (continuous cultivation of spirulina for at least 12 hours at 4°C) induced the expression of desaturase *desD* and the accumulation of ɣ-linolenic acid into biomass.

Hyperosmotic stress (caused by high quantities of NaCl in nutrient medium - 20-40g/l) induced a significant increase in the quantity of lipids (up to 63%) and carbohydrates (up to 81%) in algal biomass. It was also observed an increase in the amount of lipids and carbohydrates under the action of copper ions on spirulina culture.

Actually, moderate stress (e.g., controlled periodic illumination) brings certain technological advantages, such as increased biomass production and high levels of phycobilins and carbohydrates. However, this situation should be treated with maximum precautions, because any stress is associated with a high risk of accumulation of free radicals. Where the purpose of biomass production process is not its integral use, but the extraction of certain bioactive components (e.g., lipids, phycobilins, polysaccharides), moderate stress advantages can be successfully applied as simple, cheap and efficient technological solutions.

MONITORING OF THE ADAPTIVE CAPACITY OF DIFFERENT AGE *SPIRULINA* TO OXIDATIVE STRESS INDUCED BY HYPOTHERMIA IN THE PRESENCE OF CHEMICAL STIMULATORS UNDER LABORATORY CONDITIONS

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In order to determine the impact of the inclusion of coordination compounds in biosynthetic activity of cyanobacterium *Spirulina platensis*, it was studied the change of protein content into biomass in the presence of zinc acetate under short-duration hypothermia. Hypothermic stress was induced by continued cultivation of spirulina at 4^0C over a period of 1, 2 and 3 hours. Spirulina has been subjected to hypothermic stress at the beginning of lag phase or one day culture, at the beginning of exponential phase or three days culture and at the end of exponential phase or six days culture. Spirulina was cultivated under optimum conditions in the presence of zinc acetate concentration of 15 mg/l. The compound has been added to mineral medium from the first day of cultivation. As a control sample, spirulina culture of the appropriate age was grown under optimal conditions (continuous illumination regime, temperature 28-32^{0}C) in the absence of chemical stimulator and in the presence of chemical stimulators. Hypothermic conditions were induced for spirulina cultivated in the absence and presence of zinc acetate.

As a result, it was determined that in spirulina grown in the presence of zinc acetate on the first day of cultivation, hypothermia did not alter the protein content in the first two hours of heat stress. The reduction in the protein content has been insignificant after three hours of hypothermia. In control, reducing protein content in biomass with 16% was established after the first hour and three hours of heat stress. Spirulina culture of 3 days, in the absence of chemical stimulator, reacted to hypothermia by reducing the protein content with 28% after two hours of hypothermic stress without subsequent reduction. In the culture of spirulina grown in the presence of zinc acetate hypothermia also has altered protein content after two hours of heat stress, its value being with 24% lower compared to control sample. After three hours of hypothermia, protein content in biomass was reduced by 30% relative to control.

Spirulina culture, control sample, at the end of exponential phase (day 6 of cultivation), reacting to hypothermia by reducing the protein content with 15% after the first hour of hypothermic stress and 13% - after a two-hours exposure to low temperatures. Protein content in biomass decreased with 21% after three hours of hypothermia. In spirulina grown in the presence of zinc acetate, at the age of six days, hypothermia significantly modified the protein content. Thus, after the first hour the protein content was reduced by 64%, after two hours of heat stress –with 55%. After three hours of hypothermia, reduction of protein content in biomass was lower by 44% relative to control.

Therefore, the most resistant to hypothermia in terms of protein synthesis was culture at the beginning of lag phase and exponential phase. The most vulnerable age for spirulina culture was the end of exponential phase. The most significant reductions in the levels of protein were determined after the first and the third hour of hypothermia. For the experimental variant of spirulina cultivation in the presence of zinc acetate, it was established that hypothermia induced over a period of three hours did not altered the protein content in spirulina biomass at

the beginning of lag phase. Spirulina, at the beginning of exponential phase, lost proteins after two hours of hypothermia. The most vulnerable has proved to be spirulina in stationary phase, which lost more than half of the protein content.

Zinc acetate from cultivation medium prevented protein loss in spirulina biomass, being in adaptation period and subject to hypothermic stress. In the case of spirulina at the beginning of stationary phase, involvement of zinc acetate in biosynthetic processes has weakened the culture of spirulina which, under hypothermic conditions, lost more than half of protein content.

APPLICATION OF MICROWAVE PRETREATMENT TECHNIQUE OF APLANOSPORES OF *HAEMATOCOCCUS PLUVIALIS* FOR ASTAXANTHIN RECOVERING

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In recent years, techniques of extracting carotenoids from algal biomass have been developed under intense research efforts, because carotenoids have many applications as agents for strengthening health and exhibit antioxidant properties. Although, synthetic carotenoids are relatively cheap and easy to prepare, natural ones are more stable and contain biologically active isomers.

Application of microwaves in extracting biocompounds from vegetable raw material is considered to be less harmful to the environment and humans. Microwave-assisted extraction of carotenoids is little investigated. Main parameters in microwave-assisted extraction are microwave power, reaction medium, duration of action, temperature and type of microalgal cell wall. There were carried out researches for application of microwave drying technique as a method of cyst cell-wall pretreatment of *Haematococcus pluvialis* with later recovering of astaxanthin.

In order to monitor the efficiency of microwave pretreatment technique, it was applied astaxanthin extractability, which is used in the analysis of the effectiveness of extraction methods. Extractability value consists of the ratio of "free astaxanthin", which is amount of astaxanthin from native biomass of *Haematococcus pluvialis* by its shaking for 1 hour with acetone and "total astaxanthin", which means its extraction in acetone under the same conditions, but treated in advance for destroying the cell wall.

There were prepared samples of *Haematococcus pluvialis* biomass, aplanospores, with 10 mg in Petri dishes with a diameter of 60 mm. There were selected two variable parameters: microwave power and exposure to their action. Extraction of astaxanthin has been carried out with the use of ethanol as a solvent, in order to preserve the extracts or its later use in preparing oily product.

By applying the microwave regime of 120 sec at 180W, astaxanthin extractability in acetone was 28% and 14% in alcohol. Under conditions of 20 sec at 180W microwave power, astaxanthin extraction has been possible only with using acetone and extractability was very low, only 11%.

Application of 540W microwave power established a dependency between the time of the effects of electromagnetic waves and astaxanthin extractability. Thus, after 20 sec, astaxanthin extractability in ethyl alcohol was 32% and 38% in acetone. Doubling the time of exposure has not enhanced astaxanthin extractability, which was 38% for extracting in ethanol and 46% in acetone. Extraction of 50% astaxanthin from *Haematococcus pluvialis* biomass was set at wave action for 60 sec. Application of microwave regime of 540W for 120 sec favored astaxanthin extractability with 84% in ethanol and 82% in acetone. Therefore, the effect of microwave drying can be also applied for damaging the cell wall of red cysts of *Haematococcus pluvialis*.

Microwave pretreatment technique (540W, 120 sec) of native biomass of *Haematococcus pluvialis* can be applied as pretreatment stage of robust cysts for astaxanthin recovering.

THE IMPACT OF COMPOUND FE(III)-ALANINE ON PHYCOBILIN SYNTHESIS IN *SPIRULINA* BIOMASS UNDER HYPOTHERMIA

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In order to determine the inclusion of coordination compounds in biosynthetic activity of cyanobacterium *Spirulina platensis*, it was studied changing the protein content into biomass in the presence of zinc acetate under hypothermic conditions of short duration. Hypothermic stress was induced by maintaining spirulina cultivation at 4^0C over a period of 1, 2 and 3 hours. Spirulina has been subjected to hypothermic stress in the early lag phase or 1 day culture for, at the beginning of exponential phase or 3 days culture and at the end of exponential phase or 6 days culture. Spirulina has been grown under optimum conditions in the presence of zinc acetate in concentration of 15 mg/l. This compound was supplemented to mineral medium from the first day of cultivation. It was used as control sample spirulina of appropriate age, grown under optimal conditions (continuous illumination, temperature 28-32^0C) in the absence of chemical stimulator and in the presence of chemical stimulators. Conditions of hypothermia were induced for culture of spirulina grown in the absence and presence of zinc acetate.

Fe (III) alalinate has stimulated the accumulation of phycobilins during the exponential phase of spirulina grown under optimum conditions. By the end of exponential phase, phycobilin content was with 25% higher compared to control.

In spirulina, grown under optimum conditions in the absence of Fe (III) alalinate and subjected to short-duration hypothermia, phycobilin content increased insignificantly with extending the exposure to low temperatures. The presence of Fe (III) alalinate caused the reduction of phycobiliprotein content in spirulina biomass during the action of low temperatures. In spirulina culture, at the beginning of lag phase, grown in the presence of [Fe(III)-ala], hypothermic conditions have changed essentially phycobilin content in biomass, which were reduced by 21% after the first hour of heat stress and 1.6 times after three hours of exposure to 4^0C.

Phycobilin content decreased of 4.3 times in spirulina culture in exponential phase and subjected to hypothermic stress for one hour. Two hours of hypothermia resulted in decrease of 2.9 times of phycobilins in biomass, and exposure of spirulina to further heat stress reduced by 3.7 times their concentration. In spirulina biomass in stationary phase that has been cultivated in the presence of compound Fe (III) alanine, one hour of hypothermia caused a decrease of 5.4 times of phycobilin content. While three hours of hypothermic stress caused a more moderate reduction, 3.2 times, of phycobilin content in spirulina biomass. Therefore, stimulation effect of Fe (III) alalinate on phycobilin synthesis was annihilated by extreme temperatures. The most vulnerable has proved to be the culture of spirulina in exponential growth phase and also stationary phase that significantly lost the content of phycobilins after the first hour of hypothermic stress.

Fe(III) alalinate from cultivation medium did not alter phycobiliprotein synthesis in spirulina in adaptation period, which being subjected to hypothermic stress does not change their content. In the case of spirulina at the beginning of exponential phase and stationary phase, iron compound involved in vital processes reorienting biosynthetic activity of spirulina, which under hypothermic conditions, reduced phycobiliprotein content after the first hour of exposure to low temperatures.

Green Biotechnology

Agricultural Biotechnology

Environmental Biotechnology

EFFICIENCY OF NEW SORBENTS BASED ON CHITOSAN FOR THE REMOVAL OF HEAVY METALS FROM RESIDUAL WATERS

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Due to the fact that we assist to a continuously increasing concentration of heavy metals in waters, it imposes to take concrete and efficient measures at the level of the pollution from the different types of industry, laboratories. Removal of heavy metals from the residual waters constitutes an important aspect that requires efficient and, preferable, reusable sorbents. Novel macroporous composite sorbents were used in this work consisting of a combination between the chitosan (CS) and a synthetic polycation, poly(vinyl amine) (PVAm). The effect of different parameters such as: contact time, initial concentration of solutions, sorbent mass, pH value and temperature on the adsorption capacity of the sorbents was investigated. The sorption kinetics data were well described by pseudo-first order kinetic model, and the sorption equilibrium data were well fitted by the Sips, Langmuir and Temkin isotherms. The positive values of enthalpy confirmed the endothermic nature of the adsorption and the negative values of Gibbs free energy suggested the spontaneity of the adsorption process. As a result of desorption studies, it was found that the recovery of sorbent can be successfully done using a solution of NaOH 0.1 M.

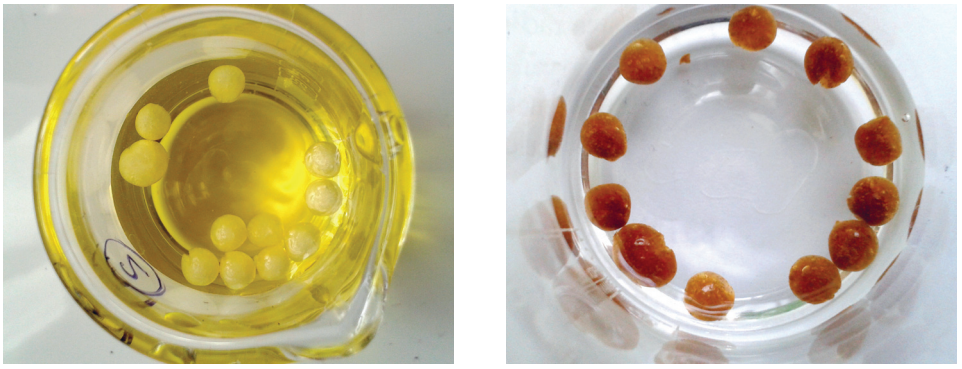

Fig. 1. Sorbent before and after adsorption process.

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SCREENING OF BACTERIA PRODUCING EXTRACELLULAR HYDROLYTIC ENZYMES FROM MANGROVE SEDIMENTS

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Mangroves are productive ecosystems, which contribute to the well being of different organisms. They are halotolerant small shrubs or trees on which the life of different species of birds, animals, crustaceans and molluscs are depended upon. Mangroves play an important role in maintaining the natural environment and different communities by providing resources like food, medicine and helping in mineral cycling, water purification, soil erosion prevention and minimizing the impact of natural disasters. The microorganisms found in mangroves are responsible for mineralization, biogeochemical cycling of nutrients and contribute significantly to the transfer of energy among various trophic levels. Most of these microbes and strains thereof produce large number of enzymes capable of hydrolyzing, oxidizing or reducing metabolic reactions. Cellulase is a type of hydrolyzing enzyme extracted from microorganisms that has applications in food, pharmaceutical, detergent and paper industries. The screening of bacteria was done from the mangrove in Payyanur region, Kerala. The physicochemical characteristics of the soil were analyzed, including soil type, temperature, pH, conductivity, organic carbon, phosphorous and potassium. Hydrolytic enzymes have a wide variety of applications in different industries such as food, pharmaceutical and textile. Four different colonies were obtained on zobell marine agar media through serial dilution plate count spread plate technique. The colonies were named as MSB-1, MSB-2, MSB-3 and MSB-4. We have examined their ability to produce hydrolytic enzymes, such as catalase, oxidase, amylase and protease, which were qualitatively analyzed in these four isolates. Hydrogen peroxide, Kovac's oxidase reagent were used for catalase and oxidase assays, respectively. For amylase enzyme, isolates were cultured on starch agar medium and for protease on skim milk agar medium and incubated at 30°C for 48 hours. All four species of bacteria showed positive results towards catalase and amylase activity and also showed negative result for oxidase enzyme activity. All are capable of producing protease enzyme, except MSB-4. The colonies will be identified through molecular techniques for further study.

EFFECT OF PLANT GROWTH PROMOTING BACTERIA (PGPB) ON COPPER TOXICITY REDUCTION IN GRAPE SEEDLINGS

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Copper (Cu) is one of the heavy metals that are necessary for the growth of plants, but at high concentrations has a toxic effect. Toxicity of copper, that can occur due to the repeated use of containing this heavy metal fungicides, in many countries is a problem of both agricultural and ecological importance. Due to necessity of multiple use of Cu-containing compounds for combating powdery mildew on perennial plantations significant amount of Cu is accumulated in the soil and plant organs. There are few ways to reduce the toxicity of copper. One of the most promising is the use of plant growth promoting bacteria and trace elements that contribute to improving plant nutrition. For this scope suspension of 3 strains of bacteria (*Agrobacterium radiobacter, Pseudomonas putida X, Bacillus subtilis L),* applied alone and together during the planting of rooted vine cuttings of cv. Victoria, and foliar application of the half of trace elements complex Microcom-VA recommended dose, were used. The experiment was performed on the growing platform of IGPPP MECR RM. Vine cuttings were rooted in water, after that planted in plastic poth with 11 kg of soil. Cu was added to the lower layer of soil in pots as $CuSO_4 \cdot 5\ H_2O$ during the planting of cuttings (1200mg Cu per 1kg soil). Different experimental variants were set: Control (without Cu), Cu alone and Cu together with suspensions of bacteria and Microcom-VA. Content of proline and photosynthetic pigments, growth and maturation of the shoots, biomass of roots were determinated.

After three months of growth the content of proline, that is a metabolic compound commonly involved in stress response, notably increased in leaves of plants grown under the surplus of Cu (136 % to the control). In variants with Cu and addition of bacterial suspension in soil and foliar treatment with Microcom-VA decreasing trend of proline content in leaves was observed. At the beginning of September, when the plant growth process ceased and the shoot maturation began, the proline content in leaves decreased, but compared to control the same trend occurred. Reduction of the proline amount in the leaves in presence of bacteria may be due to bacterial activity in contrasting the oxidative stress, increasing hydration of the leaves, producing biologically active substances, and reducing the amount of a stress-related molecule as ethylene in plant tissues. Moreover, the analysis of total chlorophyll content and chlorophyll fluorescence parameters revealed a higher photosynthetic efficiency of plants grown with the addition of the suspension of bacteria and Microcom-VA with respect to control.

Growth and maturation of the shoots under the surplus of Cu in soil have been slightly lower than control, while they significantly increased in all variants with addition of bacterial suspension and Microcom-VA. The weight of root biomass under the Cu surplus in soil was not reduced significantly. At the same time addition of the suspension of bacteria stimulated the root growth in all variants. It is worth highlighting that under these treatments an enhanced growth of small roots, which play a major role in plant nutrition, occurred.

The presented data show the possibility of mitigating the toxic effect of excess copper in the soil on the growth and development of grapes seedlings by improving the nutrition conditions of plants supplying *Agrobacterium radiobacter, Pseudomonas putida X, Bacillus subtilis* L and trace elements.

MOLECULAR DETECTION OF *CAMPYLOBACTER*, *BRUCELLA* AND *LEPTOSPIRA* SPP. IN BREEDING BULLS OF PUNJAB, PAKISTAN

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Brucella, *Campylobacter* fetus and *Leptospira* are main veterinary pathogens that cause severe reproductive problems in bovine around the world. Genital brucellosis, campylobacteriosis and leptospirosis are infectious venereal diseases that lead to huge economic loss by causing abortion in bovine. All three pathogens can be diagnosed with several serological methods, but various aspects during diagnosis can be responsible for false negative and positive results. Single polymerase chain reaction (PCR) and direct microbial isolation tools which are laborious in practice have been well demonstrated for the screening of these infections. In aim to improve direct diagnostic methods, a multiplex PCR has been developed with high sensitivity and simplicity using one hundred and sixty six (166) samples of Nili Ravi bulls for the screening of three venereal diseases in one single reaction. Results of our multiplex PCR assay were completely consistent with traditional monoplex PCR method using newly designed and reported primers. Moreover, the multiplex PCR we established may be useful as a complementary tool in routine diagnosis for detection of these three genital diseases and revealing epidemiological facts about abortion measures in bovine.

Keywords: Abortion, Brucellosis, Campylobacteriosis, Leptospirosis, Multiplex PCR, Nili Ravi

STUDY OF CHEMISTRY OF CR(VI)/CR(III) BIOSORPTION FROM BATCH SOLUTIONS AND ELECTROPLATING INDUSTRIAL EFFLUENT USING CYANOBACTERIUM *SPIRULINA PLATENSIS*

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The biosorption of Cr(III)-Cr(VI) from aqueous solutions on dry *Spirulina platensis* biomass was tested under laboratory conditions as a function of pH, initial metal ion concentration, biomass dosage, time and temperature. Optimum adsorption pH values of Cr(III) and Cr(VI) were determined as 4.0 and 2.0, respectively. The Langmuir adsorption isotherm model fitted well the sorption equilibrium of the experimental data obtained for Cr(III), while Freundlich isotherm fitted better data obtained for Cr(VI). The kinetic data were best described using the pseudo second-order kinetic model (R^2> 0.99). The adsorption process was exothermic and the values of thermodynamic parameters of the process were calculated. *Spirulina platensis* biomass was also used as a biosorbent for the removal of Cr(VI) from electroplating industry effluent as a function of biosorbent dosage and contact time.

AFFINITY OF *IRIS* × *HOLLANDICA* TO ARBUSCULAR MYCORRHIZAE DURING ESTABLISHMENT IN THE FIELD

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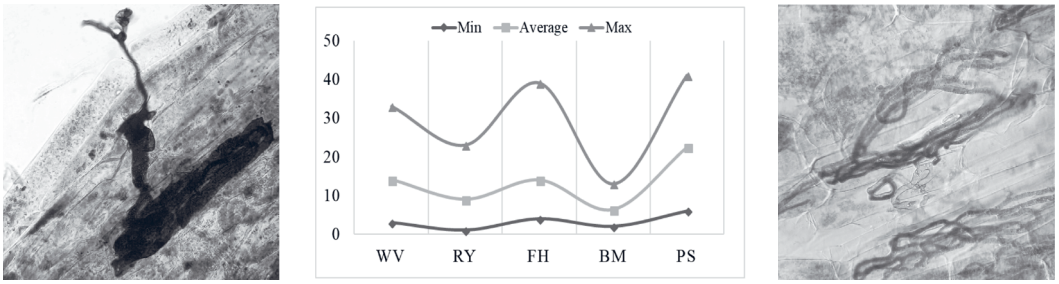
Ornamental geophytes such as *Iris* × *hollandica* have a short period of time available to accumulate nutritional resources before entering dormancy shortly after flowering. This aspect can have a strong influence on quality of bulbs, which is critical for obtaining marketable cut flowers. Arbuscular mycorrhizae (AM) are known for enhancing plant nutrition especially in phosphorus, as well as increasing plant resistance to biotic and abiotic stress. Still there is lack of studies for geophytes about their susceptibility to root colonization in field conditions.

The aim of this study was to identify the colonization susceptibility of different Dutch iris genotypes to AM naturally occurring in soils from Cluj County – Romania. Obtained results are helpful to recommend the cultivation only of cultivars with highest capacity to benefit from root symbiosis with endophytes. Also, low colonization susceptibility might limit the application efficiency of commercial bioproducts containing arbuscular mycorrhizae in given pedo-climatic conditions.

Iris × *hollandica* bulbs belonging to five cultivars: 'White Van Vliet' (WV), 'Royal Yellow' (RY), 'Frans Hals' (FH), 'Blue Magic' (BM) and 'Purple Sensation' (PS), were planted in Agro-Botanical Garden UASVM Cluj-Napoca. After 12 days (4th - 16th April 2018) root samples were collected and stained using NaOH, ink and vinegar method. Stained samples mounted on glass slides were observed under microscope at magnification 100× - 400×. The number of AMF entry points was counted for each 1 cm long root segment.



a *b* *c*

Fig. 1. AMF root entry point 400× (a); number of AMF entry points for 1 cm long segments (b); AMF hyphae coils in roots of *Iris* × *hollandica* after 12 days since planting the bulbs (c)

AM structures were intense stained and easy to observe (Fig 1.a). No arbuscules, spores or vesicles were present but sometimes intraradical hyphae were seen already being well spread between root cells. Average number of entry points per random 1 cm long root segment varied among cultivars (Fig. 1.b) with highest values per root segment around 50. Frequency of entry points along first 3 cm of root length from apex, increased from distal area towards proximal

segment, most likely influenced by maturity stage of cortical cells. Hyphopodium of varying morphology and first intraradical hyphae indicated the entry points, while in some segments extensive coiling under entry points was also visible (Fig. 1.c). The lowest AM affinity was identified in 'Blue Magic' which also presented thickest roots while the highest affinity was identified in 'Purple Sensation'.

AM colonization susceptibility during first stages of rooting lacks uniformity among studied *Iris* × *hollandica* cultivars, with potential influence on nutritional status of plants and effectiveness of commercial inoculants. Cultivars with observed high affinity for native *Glomeromycota* fungi are recommended for cultivation in Cluj county area.

MYCORRHIZAL DYNAMICS IN ROOT OF URBAN *TRIFOLIUM* GENUS

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In current century, there is an increased interest in urban ecology, in the understanding the role of microbial soil diversity for the installation and coexistence of plants. The city is an ecological complex where the atmosphere is characterized by the mitigation of climatic excesses during the winter, the accumulation of heat during summer, the reduction of ultraviolet radiation and the alteration of the chemical properties with the accumulation of pollutants emitted by different sources. Factories, transport of waste, increased levels of urban noise, human presence all act as pressure elements on urban ecosystems.

In cities, the role of ecosystems is assigned to parks, in an attempt to create habitats similar to those of nature. Inside these areas, arbuscular mycorrhizal fungi have the role of increasing the above-ground productivity by transferring nutrients into the roots of their host plants. These microbial processes contribute to plant growth and soil fertility, which are essential to the efficient functioning of ecosystems. Legumes are an important functional group because they can form a tripartite symbiosis with nitrogen-fixing bacteria and fungi mycelium to produce phosphorus. Virtually all leguminous plants are mycorrhized, as a symbiotic supplement with bacteria that produce nodules.

Our study was focused on identification of mycorrhizal structures in the root and appreciation of mycorrhizal colonization in the genus *Trifolium*. The plants were collected from the flora of three parks in the city of Cluj-Napoca. The collection period was autumn-winter 2017, in order to see the symbiotic level during vegetative rest periods. Root samples were taken at a distance of 5 m from the tree stem, to establish the potential for fungal link of their root systems with leguminous and to highlight the wood wide web connections.

The evaluated mycorrhizal structures are the number of mycorrhizal fragments, frequency, intensity, arbuscularity and colonization degree (Table 1.). Frequency of colonization is more influenced by the ecosystem than the distance from the tree. As an opposite phenomenon, colonization intensity at 1m distance from tree is 40%, and at 5m 25%. The relationship between frequency and intensity is loose, mycorrhizal development acting opportunistic according to the sampling area. Arbuscules and colonization degree are closely related, the mycorrhizal system having a gradual development in the roots of the analyzed plants. The low value of the frequency negatively influences the colonization degree, the intensity not being able to compensate for this depreciation in root system.

Table 1. Descriptive statistics of mycorrhizal parameters.

	Mean	**Minimum**	**Maximum**	**F**	***p***
Mycorrhized fragments	28,30	20,00	30,00	8,72	0,001
Frequency %	94,33	66,67	100,00	8,72	0,001
Intensity %	32,01	0,83	92,50	8,58	0,001
Arbuscularity %	19,95	0,24	59,57	6,84	0,004
Colonization degree %	31,66	0,58	92,50	8,44	0,001

An interesting case was the identification of calcium oxalate crystals deposits in an intriguing variety of forms. This is an interesting point for future studies on how mycorrhizas transfer excess nutrients from urban soils to plants and how plants store them.

Mycorrhizal systems are present in urban legumes with high colonization values during vegetative rest. Hyphal connections provide an extensive network for nutrient exchange with other plants present in the ecosystem.

ENTOMOPATHOGENIC BIOPESTICIDE IN PROTECTING *LEPIDOPTERA* DIVERSITY IN ORGANIC FARMING

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Biopesticides are mass-produced, biologically based agents used for the control of plant pests. Biopesticides are being used on increasing scales and there is considerable interest in their potential as alternatives to conventional pesticides. The agricultural biologicals market was valued at USD 6.75 billion in 2017 and is projected to grow at a CAGR of 13.80% from 2017 to reach USD 14.65 billion by 2023. This market has been gaining prominence among farmers to improve the agricultural yield with natural methods and curb excessive usage of chemical-based pesticide application. Factors such as increased demand for sustainable agriculture & organically produced food and promotions by governments for the adoption of agricultural biologicals are projected to drive the agricultural biologicals market growth.

In this review on entomopathogenic microorganism we discuss how baculoviruse host range and pathogenesis have contributed to their inherent safety for non-target organisms.

In republic Moldova, there are several chemical insecticides available and authorized for the current regulation of *H.cunea*. Wan et al. (2014) reviewed control strategies in its native range of Japan and Asia and pointed out, that regulation in parks, green belts or nurseries relies mainly on chemical insecticides, such as pyrethroids. But due to the large-scale application of broad-spectrum insecticides, *H.cunea* has already developed some level of resistance in Moldova. Furthermore, frequent applications of pyrethroids and neonicotinoids may cause severe negative effects on organisms that provide ecosystem services including pollination and natural pest control (EASAC 2015a).

Our investigations were focused on biological control methods with the intention of an easy and quick practicability and the aim of making chemical treatments avoidable, since their use is problematic in both planted and natural occurring. Biological control methods are of great advantage for regulating harmful lepidopteran pests and seem to be appropriate and promising for the eco-friendly regulation of *H.cunea*. Biological control comprises the use of biological plant protection products based on virus, bacteria, fungi, natural material; the use of pheromones, plant strengtheners and beneficial as well as general principles, with the aim of reducing the population density of the pest organism. Advantages are the specific effects on targets and thus only slight impairment of non-target organisms; insignificant influence on ecosystems and no long-term effects on soil, water and air; little or no relevant residual traces occurring in plants as well as no waiting for bystander. In addition, biological control methods can close gaps in circumstances where chemical plant protection products may not be used. Furthermore, they might benefit the natural regulation of field populations by the preservation of natural enemies.

Our researches show the difference between the parameters of biological activity of biological mass obtained on the different days from the infection with baculoviruses. There are not noticed any substantial differences in biological activity in the case of viral suspension with the same concentration (10^8 pol /ml). Good results were registered at the analysis of lethal time necessary for obtaining a death rate of 50% of larvae (TL_{50}). That parameter has minimal

value the first 5 days from infection. In the terms of that aspect, biological mass obtained from dead larvae after these days is characterized by parameters specific to wild strains obtained from natural conditions, that aspect induces the difference in biological activity of biological mass obtained from dead larvae on different days of infection and denotes the possibility of application of that measure in the process of improving bacterium strains applied for elaboration of entomopathogenic insecticides. Other authors also have confirmed the results of the investigations.

EFFICACY OF *PSEUDOMONAS AUREOFACIENS* CNMN-PB-05 AS A BIOLOGICAL CONTROL AGENT AGAINST *ERWINIA AMYLOVORA* ON APPLE FLOWERS

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Plants are host to a large amount of pathogenic bacteria. Fire blight, caused by the bacterium *Erwinia amylovora*, is an important disease in *Rosaceae*. The principal and most susceptible hosts are in the sub-family *Pomoideae* of the family *Rosaceae*. The following plants are considered as important hosts, from both economic and epidemiological points of view: *Amelanchier alnifolia*, *A. canadensis*, apples, C*haenomeles* spp., *Cotoneaster* spp., *Crataegus* spp., *Cydonia* spp., loquats, medlars, pears, *Pyracantha* spp., *Pyrus amygdaliformis*, *Sorbus* spp., *Stranvaesia davidiana*.

The ability to release products with antimicrobial activity is the major mechanism by which pseudomonads suppress pathogens. *Pseudomonas* is a diverse genus known for their ubiquity in the environment and production of secondary metabolites. Some pseudomonad strains are well-suited to be biocontrol agents, producing a wide range of bioactive metabolites. The general antibiotics produced by *Pseudomonas* include phenazine derivatives, pyoluteorin (Plt), pyrrolnitrin (Prn), hydrogen cyanide (HCN), 2,4-diacetylphloroglucinol (DAPG) and insect toxin.

The present study confirm the potential of *Pseudomonas aureofaciens* CNMN-Pb-05 strain to be used as active ingredient of microbial biopesticides for fire blight control that could be eventually extended to other plant bacterial diseases.

Experiments were done under controlled environmental conditions on detached flowers, in order to determine the effect of *Pseudomonas aureofaciens* CNMN-Pb-05 against *E. amylovora* infections. The plant material was obtained from a commercial orchard near Chisinau, and transported to the laboratory under refrigeration, and used before 24 h. Individual flowers were maintained with the cut peduncle submerged in 10% sucrose solution. Flowers were sprayed with suspension of *Pseudomonas aureofaciens* CNMN-Pb-05 at 1×10^7 CFU ml^{-1} (0.4–1 ml per flower) using a micro sprayer. All materials were introduced in plastic boxes in a controlled-environment chamber at 25 °C (±1°C), high relative humidity, and 16 h of light - 8 h dark photoperiod. After 24 h, the hypanthia of flowers were inoculated with 10 µl of a suspension of *E. amylovora* PD 4072 at 1×10^7 CFU ml^{-1}. The inoculated plant material was again incubated under the above mentioned conditions for 5 days. The experimental design consisted of three replicates per treatment with per replicate. Controls treated with water and inoculated with the pathogen were included. Incidence of infections was determined at 5 days. Results were expressed as cells or CFU per blossom using Fisher's least significant difference test ($P\leq0.05$).

The installation of *Pseudomonas aureofaciens* CNMN-Pb-05 strain on detached blossom before the inoculation with *E. amylovora* (preventive treatment) allowed an efficient control of disease (98%), with an incidence that never exceed the level 3.2%, but for curative treatment the incidence increased to 38.4% and the efficiency was 57.6%.

Under field conditions, *Pseudomonas aureofaciens* CNMN-Pb-05 applied twice (10 and 75% bloom time) at 10^7 CFU/ml reduced significantly the incidence of fire blight by 69.2%. The antagonistic strain was able to reduce significantly the incidence of infections of *E. amylovora* compared to non-treated control, where the severity of disease attack was 1.33%.

Results from this work illustrate that *Pseudomonas aureofaciens* CNMN-Pb-05 strain was able to control efficiently fire blight in apple trees under the conditions of the arias of Moldova.

VIRULENCE OF *BEAUVERIA BASSIANA* AGAINST PEA LEAF WEEVIL *SITONA LINEATUS* L. (COLEOPTERA: CURCULIONOIDAE) A NEW STRAIN FROM THE REPUBLIC OF MOLDOVA

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Pea leaf weevil, *Sitona lineatus* L. (Coleoptera: Curculionoidae), is an insect pest that causes considerable damage to a wide range of cultivated and wild *Fabaceae* species. Both life stages of *S. lineatus* are considered to be harmful, adults feeding on leaves produce severe defoliation and larvae feeding on root nodules reduce nitrogen fixation for the plant. Highest fertility of *S. lineatus* was noticed on *Pisum sativum* L. and *Vicia faba* L. Due to species high reproductive potential, migratory behavior and cryptic larval habit, management of pea leaf weevil is a challenge. Existing pest management strategies in the Republic of Moldova relay mostly on the use of chemical pesticides. Also, issues associated with pest resistance to pesticides, effects on non-target species and impact to human health lead to a critical need in developing new solutions for pest control. In this regard, biological control agents are an efficient alternative to chemical pesticides, as a tool against insect pests that can support and ensure the food safety. The first step and the most significant criteria for effective use of microbial control agents in biological control is characterization of isolated entomopathogenic strains in terms of their virulence toward a target pest and identification of highly virulent strains.

The aim of the present research was to evaluate the virulence of the newly isolated local strain of *Beauveria bassiana* Sl1/6 against adults of pea leaf weevil. In bioassay test, alfalfa was used as diet. Five serial dilutions of fungal strain in sterile distilled water were prepared to perform the test. By 1 ml of each dilution was spread on sterile filter paper in Petri dishes and 10 adults of *S. lineatus* were placed in each per Petri dish. Sterile distilled water was used as control. After inoculation insects were transferred in net cages with fresh alfalfa and kept at 25±1 °C and photoperiod of 14 h. Insect mortality was recorded daily, all dead insects were removed from cages. The most effective of the tested concentrations for *S. lineatus* proved to be 0.969×10^6 spores/ml causing 100% mortality on the day five after treatment. The virulence of the strain expressed in LC_{50} values calculated according to Spearman-Karber formula represented 1.127×10^4 spores/ml. These results show that *Beauveria bassiana* strain Sl1/6 is virulent to pea leaf weevil and has potential as a biological control agent in leguminous crops pest management programs. The *B. bassiana* Sl1/6 strain was placed in National Collection of Nonpathogenic Microorganisms, and patented (Patent no. 4560 published in The Official Bulletin of Intellectual Property (BOPI) No. 4 from April 2018).

This is the first report about bioassay against pea leaf weevil in the Republic of Moldova, using a local isolated *B. bassiana* strain. The results of the study are encouraging to continue investigation by evaluating the virulence of *B. bassiana* Sl1/6 strain against *S. lineatus* under field conditions and characterization of physiological and biochemical properties of the strain, for further development of a biopesticide formulation suitable for mass production.

ANTIFUNGAL AND PHYTOSTIMULATION ACTIVITY OF *PENICILLIUM* FUNGI

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Mycelial fungi are successfully used in contemporary biotechnology as producers of bioactive substances. A special role belongs to them in the production of biopreparations for agricultural use (fungicides, herbicides, insecticides). Trihodermin bioproduct (based on *Trichoderma* genus), vermiculite and funiculosum (*Penicillium* genus), etc., are used as agricultural plant phytostimulators and for the treatment of plants affected by various pathogens.

The purpose of the research was to investigate the antimicrobial and phytostimulating properties of some fungal strains of the *Penicillium* genus.

Strains *Penicillium funiculosum* CNMN FD 11 and *Penicillium verrucosum* CNMN FD 19 were studied. The antifungal properties of the micromycetes strains were studied according to the diffusion method using the agar blocks. The following phytopathogens served as test-culturess: *A. niger, Alternaria alternata; Botrytis cinerea; Fusarium solani; Fusarium oxysporum; Fusarium graminearum.* For studying the phytostimulatory properties, triticale seeds Ingen 93 and wheat seeds "Arnautca" were used. Metabolites were obtained in the submerged cultivation of the studied strains in the medium consisting of (%): glucose 4.0; $NaNO_3$ - 2.0; K_2HPO_4 - 2.5; $MgSO_4x7H_2O$ - 1.0; $FeSO_4$ x $7H_2O$ - 0.01; yeast extract 1.5; pH 6.2, under continuous stirring conditions (160-180 r.p.m.) at 28-30° C for 6 days. The culture fluid (the metabolites) was separated from the biomass by filtration, and then diluted with water in a ratio of 1:100; 1:200; 1:300; 1:400; 1:500. Triticale and wheat seeds were soaked in aqueous suspension of metabolites of the strains studied with these concentrations for 2 hours (consumption standard - 0.1 l / kg seed), then placed in Petri dishes on filter paper wetted with distilled water. The boxes were inserted into the thermostat for growth at 24° C for 7 days. On the 3rd day of cultivation the germinating activity was determined, and on the 7th day the final seed germination. After 4 days of cultivation, the body parameters of the plantlets were determined: the number of roots in a plant, the average length of the roots and shoots, the root length, the gross and dry weight of the roots and the plants. As a control group served the wetted seeds in distilled water.

It has been established that *P.funiculosum* strains CNMN FD 11 and *P.verrucosum* CNMN FD 19 show antagonism against all the phytopathogens tested. The diameter of the phytopathogen inhibition zones ranges from 20 to 26 mm.

In the treatment of triticale and wheat seeds with fungal metabolites, the most effective one was the concentration of 1:300. Thus, in this variant (1:300) germinating energy and germination of triticale and wheat seeds under the influence of metabolites of *P.funiculosum* strain CNMN FD 11 increased by 6-10% in comparison with control variant. The number of roots in a plant increased by 10-23%, the main roots length and the average length of the roots increased by 2 times, the average length of the plantlets increased by 1.5 times. When treating the *P.verrucosum* CNMN FD 19 metabolites, germinating activity and germination of triticale and wheat seeds increased on average by 10-17%, the number of roots of a plantlet increased by 10-31.5%, the length of the main roots and the average length of roots and plantlets has increased by 2.5 times.

As a conclusion, we estabished that the treatment of triticale and wheat seeds before sowing with the aqueous solution of *P.funiculosum* strain CNMN FD 11 or *P.verrucosum* CNMN FD 19 in a ratio of 1:300 stimulates the germination of the seeds, the growth of the roots and plantlets. It subsequently contributes to accelerating the beginning of crop maturation as well as stimulating their productivity.

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF STREPTOMYCETES FROM MOLDOVA SOILS

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Antibiotics are known to be natural substances originated from microorganisms, plants, animal tissues or can be artificially synthesized. Of the extremely diverse microbial world, actinobacteria are the number one producers of antibiotics. The largest antibiotic-producing genus in the microbial world discovered so far is the genus *Streptomyces*. The number of antimicrobial compounds reported from the species of this genus increased each year almost exponentially. Streptomycetes can also synthesize a wide range of other biologically active substances, which stimulate growth and development of plants and animals.

Here we report the strains of the genus *Streptomyces: Streptomyces* spp. 9, 33, and 66, capable to suppress the growth of plant pathogenic bacteria and fungi.

The strains were isolated from soil samples of the central zone of Moldova: 1) The strains 9 and 33 – from low-humic carbonate chernozem (humus, 2.4-2.5%) after cultivation of the maize monoculture grown without fertilizers and pesticides, and 2) the strain 66 – from heavy-loam typical chernozem (humus, 2.6%;). Antimicrobial activity was determined by a disk-diffusion method.

The isolated strains were identified in the All-Russian Collection of Microorganisms (VKM). To identify the strains, their 16S rRNA gene fragments were sequenced, the similarity levels with the most closely related type strains of actinomycete species were estimated. Then, the cultural and morphological characteristics of these strains were determined and compared with those of the closest relatives of the target strains. According to the data obtained, strain 33 was preliminary identified as *Streptomyces plicatus*, while strains 9 and 66 were supposed to represent two novel species within the genus *Streptomyces*.

Strains of *Streptomyces* sp. 9 and *Streptomyces* sp. 66 were found to suppress a wider range of plant pathogenic fungi (10-11 strains) as compared with *S. plicatus* 3 (3 fungal strains). In particular, *Streptomyces* sp. 9 completely inhibited the growth of *Sclerotinia sclerotiorum* and actively inhibited the growth of the two *Fusarium* species, *F. oxysporum* and *F. graminearum* (diameters of inhibition zones were 34.0 and 28.0-29.0 mm, respectively), as well as *Rhizoctonia solani* and *Thielaviopsis basicola* (inhibition zone reached up to 29.0 mm). Strain *S. plicatus* 33 could suppress *Alternaria alternata* and *F. graminearum*.

The comparison of antimicrobial activity of the above strains against plant pathogenic bacteria showed that only *Streptomyces* sp. 9 suppressed the growth of all four test-strains belonging to *Clavibacter michiganensis*, *Xanthomonas campestris*, *Erwinia carotovora,* and *Agrobacterium tumefaciens* (inhibition zones ranged from 14.0 to 30.0 mm). *Streptomyces* sp.66 suppressed the growth of the three test-cultures, namely, *C. michiganensis* (26.0 mm), *A. tumefaciens* (18.0 mm), and *E. carotovora* (18.0 mm), while *S. plicatus* 33 was active only against *C. michiganensis* (23.0 mm) and *X. campestris* (11.0 mm).

Thus, the new strain *Streptomyces* sp. 9, can be considered as a potential producer of antimicrobial compounds used against widely spread in Moldova phytopathogens.

DIRECTED SYNTHESIS OF EXOPOLYSACCHARIDES IN NEWLY ISOLATED CYANOBACTERIUM *NOSTOC HALOPHILUM* HANSG.

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Exopolysaccharides play a significant biological role in the life of microorganisms, which consume significant amounts of carbon and energy for their production. Exopolysaccharides act as agents of adhesion and facilitate the interactions of cellular associations of microorganisms, including cyanobacteria. Another important function of these substances is the protection from unfavorable physical and chemical factors, or virus and bacteria attacks. Moreover, the exopolysaccharides production presents a way for cyanobacteria to ensure their survival in nutrient-poor environments.

Polysaccharides are of great interest to biotechnologists due to the wide range of potential applications in the pharmaceutical, food and cosmetics industries etc., serving as emulsifying agents, as well as, viscosity, dispersion and chelating substances.

Investigation of the new potential polysaccharides sources is one of the priorities for modern biotechnology. The purpose of this paper was to investigate the cyanobacterium *Nostoc halophilum*, as well as the action of some physico-chemical factors on the accumulation of exopolysaccharides.

During the investigations of the natural ecosystems, a strain of cyanobacteria that actively vegetates in the soil in the Cogâlnic river meadow Cimislia, R. Moldova was isolated and then obtained a pure culture. This strain is characterized by ribbon trichomes that create dry black crust on the ground. In a pure culture, the strain of cyanobacterium *Nostoc halophilum* CNMN-CB-17 has been stored in the National Collection of Microorganisms of the Institute of Microbiology and Biotechnology.

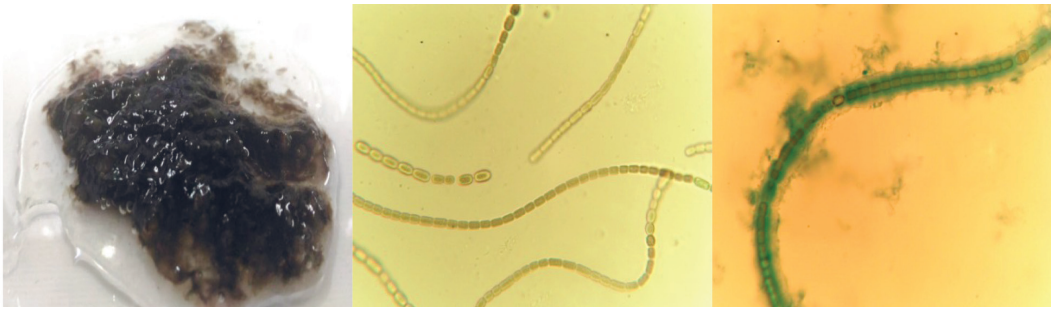

Figure 1. The general aspect of the *Nostoc halophilum* strain (a); microscope photo of strain (b); exopolysaccharides coloured with Alcian Blue (c).

Cultivation of the strain in two illumination regimes: 1500 lx and from seventh day – 2500 lx with supplementation of 1, 2 and 3 mg/l ammonium acetate revealed that the more intense illumination and the addition of 1 mg/l ammonium acetate led to the highest exopolysaccharide content at the 14th day of cultivation (385 mg/g). With the increase of acetate concentration, the amount of exopolysaccharides was decreasing, reaching values of 179 mg/g at CH_3COONH_4 concentration of 3 mg/l.

Thus, the synthesis of exopolysaccharides by *Nostoc halophilum* was stimulated by the use of two stress factors: ammonium acetate (1 mg/l) and enhanced illumination (from 1500 to 2500 lx) at the second stage of cultivation.

OPTIMIZATION OF NUTRIENT MEDIUM FOR THE CULTIVATION OF BACTERIAL ANTAGONIST *BACILLUS SUBTILIS* CNMN-BB-09

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The aim of the research was to study the features of the growth of *B. subtilis* CNMN-BB-09 bacteria on classical nutrient media and to determine the optimal composition of nutrient medium that promotes a high number of viable cells while maintaining antagonistic properties.

Preliminary experiments showed that after two days of cultivation, the highest titer was observed on the medium MPB (4.1×10^9 CFU/ml), on the medium PD (1.2×10^9 CFU/ml), and on the medium M9 the titer was lower (3×10^8 CFU/ml). PD and MPB media are natural, whereas M9 medium is synthetic. Natural medium are not quite convenient to use for the development of culture, because of their high cost, and require the use of food and a long time for their preparation. Synthetic medium M9 is cheaper and easy to perform. Thus, the M9 medium can serve as a basis for further development of optimal medium for cultivation of bacteria *B. subtilis* CNMN-BB-09.

The first stage of research was to determine the optimal source of carbon and nitrogen. The highest titers of 2.1×10^9, 5.9×10^9, 2.4×10^9 were reached for sucrose, maltose, glucose as sources of carbon, respectively. When we used sorbitol and dulcite as carbon sources, the growth of crop was inhibited. Ammonium and nitrate salts as well as glycine were used as sources of nitrogen nutrition in experiments. The best sources of nitrogen used in experiments are salts containing ammonium ions.

The addition of some amino acids has affected the quantitative growth of studied bacteria.

Table 1. The effect of various additives on the number of viable cells

Nutrient medium	Titer of *B. subtilis* CNMN-BB-09 CFU/ml, time of cultivation, h		
	48	72	96
M9	1.3×10^8	8.8×10^8	2.6×10^9
23-(with the addition of sodium citrate)	1.4×10^9	2.3×10^9	4.8×10^9
24-(dry yeast)	5.9×10^9	4.9×10^9	4.1×10^9
25-(trypton)	7.1×10^8	1.7×10^9	5.7×10^9
26-(glycerin)	1.3×10^8	9.2×10^8	5.7×10^9
27-(the joint addition of all components)	7.5×10^9	8.9×10^9	1.7×10^{10}

Thus, on the medium with threonine, methionine, lysine, valine, histidine, leucine and arginine, the culture titer after the first day of cultivation was in the range of 1.1×10^7 CFU/ml – 5.3×10^7 CFU/ml. With the addition of tryptophan and phenylalanine, the culture titer reached only 9.8×10^6 CFU/ml and 6×10^6 CFU/ml, respectively. After two days of cultivation, titer of bacterial culture on the medium with lysine and histidine was the highest (1.6×10^9 CFU/ml). Slow growth of culture was observed on the medium with threonine and phenylalanine. After three days of cultivation, the highest titer of bacterial culture was on media with

the addition of methionine, valine and tryptophan. After 4 days of cultivation, titer reached 1.2 × 10^{10} CFU/ml on the medium with arginine. Titer decreased sharply to 8.2 × 10^{8} CFU/ml on the medium with leucine. When determining the effect of amino acids on antagonistic activity of *B. subtilis* CNMN-BB-09, it was observed that the addition of an amino acid to nutrient medium did not change the antifungal properties. In order to improve the growth characteristics and antifungal activity of culture, some additions were made into nutrient medium. These media are below numbers 23, 24, 25, 26, 27. The maximum titer of 2.6 × 10^{9} CFU/ml for *B. subtilis* CNMN-BB-09 was observed on the nutrient medium M9 on day 4 of cultivation. Whereas, on the nutrient medium 27, titer was 7.5 × 10^{9} CFU/ml after two days of cultivation, and on day 4 reached 1.7 × 10^{10} CFU/ml.

The results of the experiment to determine the antifungal activity of tested bacterial strain cultivated on modified media are presented in Table 2. Analysis of the results showed that antibiotic substances were formed on the second day of culture growth.

Table 2. The effect of various additives on antifungal activity of *B. subtilis* **CNMN-BB-09**

Nutrient medium	Radius of sterile zones depending on the concentration of bacterial suspension, mm				
	3%	1%	0.5%	0.2%	0.1%
M9	0 (3)	0(0)	0(0)	0(0)	0(0)
23	10(3)	8(2)	8(2)	7(3)	7(3)
24	14(7)	15(6)	12(5)	10(5)	8(3)
25	15(5)	15(10)	13(10)	11(7)	10(5)
26	11(5)	9(5)	9(3)	8.5(3.5)	5.5(3)
27	17.7(8.8)	15(10)	15(10)	15(2)	12(2)

Thus, studies have shown that the optimal components of nutrient medium for cultivation of *Bacillus subtilis* CNMN-BB-09 are: dry yeast, sodium citrate, tryptone, glycerin. Therefore, the composition of optimized nutrient medium is as follows, (g/l): ammonium sulfate – 1.4, potassium phosphate single-substituted – 1.5, sodium phosphate dibasic – 2.5, sodium chloride – 0.5, glucose – 2.0, dry yeast – 4.0, sodium citrate – 0.25, trypton – 0.25, glycerin – 2.0 ml, water - the rest.

The optimized nutrient medium is designed for growing *Bacillus subtilis* CNMN-BB-09 in the laboratory and makes it possible to obtain a significant yield of viable cells in 48 hours of cultivation. In addition, whenever cultivated on the proposed medium, high antagonistic properties of studied culture are preserved.

STUDIES ON THE USE OF VEGETABLE SCRAPS

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Often, fruit and vegetable waste is generated before reaching consumers, due to programmed overproduction and unfulfillment of retailer quality standards. Each year, an estimated one-third of all food produced for human consumption is lost or wasted world-wide. Hence, wastes leade to environmental problems due to its high biodegradability, represents a loss of valuable biomass and an economic cost for companies. The solution to reduce the amount of vegetable waste sent to the landfill station is to find ways to reuse them. Different strategies for reduction, reusing and recycing of fruit and vegetable waste have been proposed globally.

In the present study, were analyzed the conditions to regrow onions *(Allium cepa L.)* from the scraps at different parameters.

Culture medium is the most important part of plant tissue culture. Culture media like as soil provides to cultures the necessary inorganic nutrients. In addition, they also provide the necessary organic compounds such as vitamins, and carbon source, which are usually produced in plants.

The soil provides a physical support for regenerated plants. The liquid medium enables explants to keep a constant maximum contact with nutrient supplies. A selective reagent may also be included in a culture medium to restrict the growth of certain cultures. Therefore, medium formulas vary depending upon the purpose of plant tissue culture. Different media may also be used during a plant tissue regrow process.

Another important function of a culture medium is creating a necessary environment for the plant grow. Thus, light and air are indispensable in photosynthesis and regeneration of plant tissues.

The thickness of the reproductive part has a great influence on the subsequent development of the plant. When the axillary bud (terminal bud or apex) is damaged, the plant grows harder. At the insignificant deterioration of the axillary, the plant grows more rapidly and more new leaves are generated.

Figure 1. Scraps of onions regrown in light and dark.

NON-EXPENSIVE CARRIERS FOR *RHODOCOCCUS RHODOCHROUS* CELLS IMMOBILIZATION

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Bioremediation of polluted environments is based on contaminant biodegradation, that is, metabolic abilities of microorganisms to transform or mineralize organic contaminants into less harmful, nonhazardous substances, which are further integrated into natural biogeochemical cycles.

Rhodococcus species are ubiquitous in pristine and contaminated environments, possess remarkable metabolic activities, can persist under harsh environmental conditions, compete successfully in complex bacterial populations, and therefore could be considered as having great potential in bioremediation applications. Upon revealing new catabolic abilities of *Rhodococcus* species and isolation of environmental strains degrading a wide range of contaminants, these bacteria have been increasingly explored for bioremediation of soils, waters, and air polluted with different recalcitrant and toxic organic chemicals.

Using microbial cells, immobilized for the purpose of biosynthesis or biodegradation reactions remains one of the most researched technologies of recent years. Numerous biotechnological processes are advantaged by immobilization techniques and therefore several such techniques and support materials have been proposed. Immobilized cells are frequently used for the biotransformation due to advantages such as the prevention of the elution of impurities from the cells, easy separation of the cells from a reaction mixture, repeated use of the immobilized cells, and enhanced stability of the cells.

For cell immobilization are used media types and methods well studied in the last two decades. However, the methods of incorporating microbial cells into different gels such as polyacrylamide gel, alginate, carrageenan, polyvinyl alcohol, are predominant. The immobilization supports are classified as inorganic (montmorillonite, zeolite, diatomite, different clays, anthracite, porous glass, activated charcoal, etc.) and organic as cellulose (DEAE-cellulose), wood sawdust, delignified sawdust etc. Inorganic supports have been selected to immobilize microorganisms because they can survive microbial degradation and are thermostable [Verma, 2010; Bayat, 2015].

The main objective of our research was to provide a simple, non-expensive and efficient carrier for the preparation of immobilized rhodococci cells. The strain *Rhodococcus rhodochrous*, deposited in the National Collection of Non-pathogenic Microorganisms of the Republic of Moldova as *Rhodococcus rhodochrous* CNMN-Ac-05, was used as a object of study. The rhodococci cells were immobilized on 1 g of solid support according to Kitova et al., 2004. The amount of immobilized cells was estimated by two different methods: indirect, or spectrophotometrically, by measuring the D540 optical density of the liquid medium before and after immobilization, and direct, by quantifying the number of viable bacterial cells (colony forming units, CFU) inoculated on Tryptic Soy Agar medium before and after immobilization. The inorganic supports, such as bentonite and kieselgur, were selected as excellent supports for adsorptive immobilization of *R. rhodochrous* cells (69.3% and 97.3% of bacterial immobilization, respectively). Readily available, low-cost organic carriers, such as shells from walnut, hazelnut, pistachio, peanut, husks from pumpkin and sunflower seeds, were tested as supports for immobilization of *R. rhodochrous* cells. The shell from peanuts as support material demonstrated a good adsorption of bacteria cells – 34.3%, while cells immobilization on the husk from sunflower seeds was very low – 6.2%.

THE USE OF BIO-FERTILIZER STRAINS OF GENUS *NOSTOC* FOR TOMATO CULTIVATION

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The application of algal bio fertilizers in the cultivation of agricultural crops is efficient and necessary, due to their significant benefits for the soil (characterized by the improvement of soil structure, the increase of water maintenance capacity and biogenic content etc.) and crop plants (by providing nutrients, stimulating seed germination, increasing productivity and improving biomass quality, protecting against pathogens, etc.). The selection of algal bio fertilizers for crop cultivation should be based on the idea of using of massively growing in the soil species. These algae species should be able to supply for a long time the soil with nutritive and biological stimulating substances for plants and soil micro biota. The most common group of algae that meets these requirements is the genus *Nostoc*, which species are frequently found in various soil types and under different conditions.

In this paper are presented the results of the study of algal bio fertilizers effects on the cultivation of tomatoes in greenhouse conditions. The research was carried out under greenhouse conditions at the company "AȚ ZIM" SRL, Republic of Moldova during spring – autumn, in 2 sectors (experimental and control) with an aria of 20 m^2 per sector. The living biomass of *Nostoc flagelliforme, N. gelatinosum and N. puntiforme,* at the dose of 6 kg / ha, was distributed equally on experimental sector 15 days after planting the seedlings in greenhouses; the additional administration of the bio fertilizers at the same dose was performed every 22 days after. The chemical fertilizer nitroamophos ($NH_4H_2PO_4$+NH_4NO_3+KCL) was administered in both sectors in the same concentration on the 40th day and on the 100th day, after the initial inoculation of the algal bio fertilizer.

During the experiments, the pH and the total nitrogen content in the soil, the height and the yield of each tomato plant have been monitored. The administration of algal bio fertilizers induced positive changes in soil properties, characterized by a slight change of pH in alkaline direction (soil pH in the experimental oscillated within the limits of 7.08-7.98, and in control sector - in the range of 6.59-7.48) and an increase in the total nitrogen soil content (0.28% - 1.31% in the experimental sector compared to 0, 28% -1, 11% in the control sector). The administration of algal bio fertilizers had a positive impact on tomato growth in the first 44 day (experimental plants had a height of 137.50 ± 4, 22 cm, and in the control sector – 133.50 ± 6.30 cm). Further the increase in height has slowed in experimental sector and at 110 day of analysis the height of the plants in the experimental sector was lower compared to the control (154, 16 ± 12.23 cm, and 164.46 ± 4.64, respectively). The administration of algal bio fertilizers had a positive effect tomato yield: 16, 33 ± 1, 48 tomatoes / plant in experimental plants, compared to 13, 53 ± 1, 22 tomatoes / plant and in the control variant. In conclusion, we can state that the application of algal bio fertilizers have a positive effect on soil properties and tomato plant growth and yield.

INHIBITION OF FUNGUS INDUCED BY BACTERIA

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Antifungal activity is a relatively common characteristic among bacteria, conferring an ecological advantage in environments which support the growth of a mixed bacterial and fungal flora. This activity has been detected by using a variety of *in vitro* methods, but not in all cases the chemical basis for this activity has been elucidated. This activity has significance in four areas: 1)development of therapeutic antifungal drugs, 2) development of plant protection agents, 3)suppression of fungal colonization: proliferation within the human body resulting in modification of the pattern of certain human clinical infections, and 4)reduction in the efficiency of isolation of fungal pathogens from clinical specimens.

In this regard, the strains of microorganisms that are used in biotechnology present a commercial value and the problem of maintaining as longer as possible of their valuable biosynthetic proprieties is permanently in the attention of scientists. The conservation of microorganisms and of their properties requires the use of efficient methods of preservation and a continuous monitoring of the effectiveness of these methods.

In our study, the antifungal activity of the strains of *Pseudomonas aurantiaca* CNMN-PsB-08, *Pseudomonas aureofaciens* CNMN-PsB-07 and *Bacillus cereus var. fluorescens* CNMN-BB-07 against the strains of fungal pathogens was evaluated, after their freeze-drying storage in the protective environment of Na succinate + 12% sucrose during the 3 and 6 years. The obtained results proved that the antifungal activity increased with the extension of the storage period. Thus, the collected data have demonstrated that after 6 years of conservation of *Pseudomonas aurantiaca* CNMN-PsB-08 and *Bacillus cereus var. fluorescens* CNMN-BB-07 strains, their antifungal activity against micromycetes *Fusarium oxysporum* and *Fusarium solani* increased: the diameter of the inhibition zone was larger by 5.0 – 4.7 mm and 6.3 – 7.7 mm, respectively, comparing with preservation period of 3 years.

The antifungal activity of all isolates has enhanced during the storage period of 3 to 6 years against the micromycetes *Alternaria alternata* and *Botrytis cinerea* which also are active pathogens of crop plants. In this case, we can observe that the diameter of the inhibition zone of the strain *Pseudomonas aurantiaca* CNMN-PsB-08 against *Alternaria alternata* and *Botrytis cinerea* was growing by 8.6 and 5.3 mm, respectively. The strains *Pseudomonas aureofaciens* CNMN-PsB-07 and *Bacillus cereus var. fluorescens* CNMN-BB-07 have manifested antifungal activity against *Aspergillus niger*, with the increasing of the inhibition zone diameter from 12.3 to 19.0 mm and from 14.3 to 15.0 mm, respectively.

The most significant increase of the antifungal activity was determined at the strain *Pseudomonas aureofaciens* CNMN-PsB-07 over the micromycetes *Botrytis cinerea*, with the inhibition zone increasing 2.03 times during the storage period from 3 to 6 years.

The use of these bacterial species in biological control would allow increasing the plant resistance to pathogens. Thus, the efficient conservation methods allowing the maintenance or the increase of antifungal properties are an important step in the biotechnological production of efficient fungicide alternatives for plant protection.

EVALUATION OF THE INFLUENCE OF EFFECTIVE MICROORGANISMS IN THE PROCESS OF BIOCONVERSION OF ORGANIC WASTES

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Obtaining organic agricultural production is a matter of global importance for society. The global environmental situation, including the regional, has worsened in the last century due to the industrialization and chemicalization of agriculture, the storage, preservation and unreasonable use of organic waste, etc.

These have resulted in pollution of the environment and its components. A special role in the improvement of the environmental situation belongs to the technology of bioconversion of organic waste using biological methods (worm cultivation technology). Effective microorganisms were discovered by Teruo Higa, professor at Ryukyus University in Okinawa (Japan) in 1980.

The technology of effective microorganisms opens new perspectives and opportunities for sustainable agriculture. It can become the basis for efficient production of organic products of plant and animal origin.

In the article are reported the results of the study of biochemical quality of biocompost obtained by using the effective microorganisms from microbial preparations „Baikal ЭМ-1" and „EM-1", in the process of bioconversion of the unfermented organic wastes.

The purpose of the research was to determine the influence of microorganisms on the processing of unfermented organic waste and the quality of the compost obtained.

For the research purpose, two microorganism concentrates "Baikal ЭМ-1" and "EM-1" were diluted in a ratio of 1:100 using unchlorinated and filtered water, having a temperature of 20-25°C and with the addition of molasses nutrient solution. The working solution was obtained by multi-stage process and subsequently used for the processing of unfermented cattle manure. For processing 0.5 tons of manure, were used 50 liters of working solution. The process of manure processing with the preparation „Baikal ЭМ-1" was performed under anaerobic conditions and with the preparation „EM-1" under aerobic conditions.

The biochemical investigations of unfermented cattle manure and compost obtained after 2 months of microorganism processing have been performed according to the methods set forth in the Standards (ГОСТ 26204-84-262013-84, 1984 and ГОСТ 26712-94, 1994) and specialized textbooks (Petuhova E.A. et al., 1989, Popov A. B. et al., 1973, Razumov B.A., 1986).

Analyzing the experimental results it was found that in biocompost obtained from the unfermented manure of cattle, subjected to the bioconversion process using preparations „Baikal ЭМ-1" and „EM-1" diminished essentially the content of ammonia, respectively with 79.68% - 70.91% and 85.09% - 70,03%, and increased the total nitrogen, respectively with 147.33% - 105.33% and 162.67% - 128.00% in comparison with the same indicators in manure samples at the initial stage.

Consequently, it has been found that use „Baikal ЭМ-1" and „EM-1" preparations has led to substantial changes in content ammonia and total nitrogen, thus improving the quality of the biocompost.

HALOTOLERANT BACTERIA WITH CELLULOLYTIC ACTIVITY ISOLATED FROM A HYPERSALINE NATURAL LAKE LOCATED IN ROMANIA

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1. Introduction

Cellulases are a group of enzymes that are used in many biotechnological processes. Since most of the enzymes synthesised by mesophilic microorganisms are unstable in industrial environments, it is necessary to direct research towards extremophile cellulolytic microorganisms because the enzymes synthesised by them are stable and active even in harsh physicochemical conditions [1]. Therefore, the aim of our research was to isolate and identify some microbial cellulolytic strains from a hypersaline lake located in Romania and to determine their optimal growth conditions.

2. Materials and Methods

Mud samples were collected from a salted water, more precisely Movila Miresii Salt Lake, located in a plain area of Brăila County, Romania (GPS coordinates: N 45°13'14.62" / E 27°38'31.58"). The halophilic microbial strains were isolated by serial dilution plating technique on moderately halophiles growth medium (MH) [2] [3]. For screening cellulase producers, the microbial isolates were grown in MH medium supplemented with varying concentrations of carboxymethyl cellulose (CMC: 0.5%, 1%, 1.5%, 2%, w/v), as sole source of carbon. In order to select the cellulolytic strains, Congo Red staining method was used [4].

Cultural characteristics of the cellulase producing strain were determined by culturing it on solid MH medium. Morphological characteristics were studied by wet mount preparations, Gram staining and scanning electron microscopy (SEM). The ability of the investigated strain to produce extracellular hydrolytic enzymes was tested on MH basal medium (without glucose and proteose-peptone) supplemented with an appropriate substrate (1% casein, 15% gelatine, 1% pectin, 0.1% Tween 80, 1% starch, 0.2% inulin) [5] [6] [7].

The molecular identification of the cellulase producing bacterial strain involved the isolation of genomic DNA, PCR amplification of the 16S rDNA gene and its sequencing.

To determine the optimal growth conditions (temperature, salinity, pH) of the cellulolytic strain, it was cultured in liquid MH medium and the absorbance (OD) was measured at 660 nm and 24h intervals using a microplate spectrophotometer (FLUOstar Omega, BMG Labtech).

Endoglucanase activity was determined by measuring the reducing sugars using CMC (2%, w/v) as the substrate. The amount of reducing sugar that was released by the enzymatic hydrolysis of CMC was estimated using the 3,5-dinitrosalicylic acid (DNS) method [8].

3. Results

Based on the cultural characteristics and colony morphology, 25 bacterial strains were isolated from the mud samples. Of these, a single strain showed the potential to degrade CMC from the culture medium. The cellulolytic bacterial strain forms round and relatively small colonies, which are characterized by mucous consistency, white-creamy colour and glossy

surface. The profile of the colonies is convex and their margins are regular. Bacterial cells are Gram positive, rod shaped and motile. The bacterial cells showed positive reactions for catalase, oxidase, caseinase and lipase.

By comparing the nucleotide sequence obtained with the 16S rDNA sequences from the GenBank database (NCBI), a similarity of 100% with *Bacillus zhangzhouensis* strain MCCC 1A08372 (NCBI Reference Sequence: NR_148786.1) was identified.

Experimental results showed that the bacterial strain can grow in a relatively wide temperature range: from temperatures below 15°C to temperatures above 45°C. Of the 6 tested thermal values (4°C, 15°C, 20°C, 30°C, 37°C and 45°C), the bacterial strain exhibited the highest growth rates at 15°C. By growing the bacterial strain on MH media with varying NaCl concentrations (0M, 1M, 2M, 2.5M, 3M, 3.5M, 4M and 5M), it was observed that it developed in the salinity range 0M - 3M. The highest level of turbidity was measured in the culture medium with 2M NaCl after 72h of incubation. The bacterial strain was able to grow in the pH range 5 - 8.5 and it grew optimally at pH 7.5, after 96h of incubation.

Regarding the endoglucanase activity of the bacterial strain, the results of the experiment revealed that the intensity of the hydrolytic process was influenced both by variations in salinity of the culture medium and by changes in the thermal values. The most significant enzymatic activity was detected in the growth medium supplemented with 3M NaCl, after 72h of incubations at 15°C. In this situation, the amount of glucose released from a volume of 0.5 mL of CMC substrate (2%, w/v) was equivalent to 2.05 mg.

4. Conclusion

The cellulolytic potential of the halophilic microbial strains isolated from the Movila Miresii Salt Lake is limited. Of the 25 isolates, only one strain demonstrated the ability to hydrolyse carboxymethyl cellulose from the culture medium, the size of the hydrolysis zone being reduced. Regarding the physiological particularities, the cellulolytic bacterial strain isolated from Movila Miresii Salt Lake is the first reported *Bacillus zhangzhouensis* strain that exhibits the ability to degrade cellulose and that demonstrates tolerance to high salt concentrations. Being stable and active at high salt concentrations, these enzymes synthesized by salt-tolerant microorganisms could play an important role in different biotechnological applications. Consequently, it is necessary to study the extremophilic microorganisms and their enzymatic profile to identify such active molecules with new structural and functional properties that can increase the efficiency of some industrial processes.

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THE FUNGICIDE EFFECT OF BIOPREPARATION BASED ON *TRICHODERMA* AGAINST AGRICULTURAL PLANT PATHOGENS

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The present research is devoted to expansion of fungicidal action of the liquid biological preparation Gliocladin-SC. This biopreparation has been included in the State Registry of phytosanitary assets and products raising the soil fertility of the Republic of Moldova under the number 08-2-0406 since 2015. The active substance in biopreparation is a fungus antagonist of phytopathogens *Trichoderma virens* Miller, Giddens and Foster, strain 3X.

The experiments were conducted under laboratory conditions. The materials used in this study were pure cultures of fungi, which are causative agents of diseases. Pathogens were extracted from affected plants, roots and seeds of wheat, sunflower, corn, soya, peas, cabbage. The extraction was carried out by methods commonly used in microbiology (N.S.Egorov, 1995). Fungicidal activity of biopreparation was determined on agar nutrient medium in Petri dishes using the diffusion method in agar (N.S.Egorov, 2004). Incubation was done under temperature convenient for pathogens. In cases when the biopreparation exhibited antifungal activity, a sterile zone has been formed between the well with the preparation and the culture of the pathogen.

As a result of the experiments, it was determined that all pathogens showed sensitivity to biological preparation to a various extent. Growth inhibition zones are shown in the table 1.

Table 1. Zones of delayed growth of phytopathogens under the action of biological preparation Gliocladin-SC

№	Pathogen	Diameter of growth inhibition zones, mm
1	*Botrytis cinerea*	32.8±1.1
2	*Fusarium oxysporum*	42.0±0.7
3	*F. culmorum*	21.0±0.7
4	*F. graminearum*	20.0±0.4
5	*F. verticillioides*	18.8±0.3
6	*F. sporotrichiella* (Bilai)	60.0±0,8
7	*F. solani*	11.5±0.3
8	*F. gibbosum*	9.8±0.7
9	*Fusarium sp.4*	4.2±0.3
10	*Fusarium sp.5*	11.0±0.4
11	*Fusarium sp.11*	11.5±0.7
12	*Fusarium sp.13*	5.2±0.3
13	*Rhizoctonia solani*	19.7±0.9
14	*Sclerotinia sclerotiorum*	45.0±1.3
15	*Thielaviopsis basicola*	5.8±0.5

Biological preparation Gliocladin-SC has a fungicidal action against pathogenic fungi, which cause *Fusarium* root rot of wheat, corn, sunflower, soybean, etc. It is active against pathogens affecting cabbage plants at all stages of development and during storage – *R.solani*, *B.cinerea, T.basicola, Fusarium sp, S.sclerotiorum.* It forms sterile growth inhibition zones under laboratory conditions. Application of biopreparation in agriculture will contribute to improving the phytosanitary situation in agrocenoses and obtaining environmentally friendly products.

THE INFLUENCE OF MAINTENANCE CONDITIONS ON THE QUALITY OF THE BULL AND ROOSTER SEMEN

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Under optimum conditions, the native semen is characterized by a determined number of atypical cells and this means a normal phenomenon. The increased deviations from the researched index may serve as a sign of reproductive system functional activity maladjustment. The exposure of animals under the influence of environmental stressors influences on all body systems and this leads to the development of a state of stress, that manifests itself in loosing the capacity of forming the protective barrier and maintaining the balance of body's internal state. Under the influence of stressors, functional changes in the activity of the reproductive system appear. Stressors influence on the spermatogenesis and also they can change the morphological structure of male sexual cells that can have a negative influence in the process of ovule fertilization.

The purpose of the researches was to study the influence of the maintenance conditions on the quality of bull and rooster semen. As object of study there were used breeding bulls of speckled-black race and roosters, including the semen collected from these animals after being exposed to stressors. There were used microscopic methods for determining the morphology of the semen, the nociceptive stressing method, the method of colouring the gametes and the method of studying the gametes acrosomal system. It was realized the analysis and classification of the pathological forms of the gametes in the bull and rooster semen. In the early stages of the study, there was evaluated the amount of the pathological spermatozoids in the rooster semen, kept in comfortable conditions.

The results proved that the pathological forms reach the level of 16.6±1.92 % in the semen. Among gamete pathologies there were observed: microspermatozoids, spermatozoids with aproximal straw, spermatozoids with twisted tail, macrospermatozoids, spermatozoids with broken heads and spermatozoids with ripped tails. The microspermatozoids and the spermatozoids with protoplasmic drop were detected in larger amount, respectively, 3.7±1.09; 3.3±0.65%. The macrospermatozoids were detected in a smaller amount (1.7±0.74). After rooster nociceptive stressing the percentage of gametopathologies has risen by 3.7 % and reached the level of 20.3±2.32%. In this case, the quantity of spermatozoids with twisted tail increased from 2.7±2.49% to 9.3±1.68%. The results of the experiments prove that the percent of atypical cells has grown to the maximum amount of 24.7±2.49% until the 45 day of the trials, but from day 61 to day 88 it was observed a totally azoospermia. A similar study on breeding bulls proved that the amount of gamete pathologies in the period before stressing was 13.2±1.2%, whereas the number of spermatozoids with normal morphological structure achieved the level of 86.8±1.2%. Already on day 17 after exposure to stressors it was observed an increase of gamete pathologies to 30.7±2.75%. The biggest changes were observed in the day 53 after the animals were stressed, so the amount of gamete pathologies reached the level of 86.7±2.01%. This quantity persists for a period longer than 55 days, after this period it lowers a little, but anyway it is maintained on a high level for a period of 140 days. Consequently, only the spermatozoids that totally preserve their morphological structure can manifest functional

activity. This determines the path of significant vital processes. Because of this, determining the amount of pathological cells is very important in developing the processes of increasing the results of animals' reproduction.

Based on the study carried out, it can be concluded that:

1) The semen of roosters that were kept in comfortable conditions has a bigger percent (16.6%) of gamete pathologies, in comparison with the semen of bulls (13.3%), also kept in comfortable conditions.

2) At the breeding bulls exposed to nociceptive stressing, unlike roosters, the maximum amount of pathological spermatozoids was observed on the 53rd day after steaming stress and that was lasted for a period of 2 months, until that was minimized.

PAENIBACILLUS PS-K-17 SPECIES AS A PROMISING BIOTECHNOLOGICAL AGENT FOR PRODUCTION OF POLYSACCHARIDES AND HYDROLYTIC ENZYMES

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The genus *Paenibacillus* belongs to the family *Paenibacillaceae* and includes nearly 200 representatives of bacteria widely distributed in nature. In 1993 based on the phenotypic, phylogenetic (analysis of nucleotide sequences of 16S rRNA gene) and chemotaxonomic data, some bacterial species from genus *Bacillus* were transferred to a new group described and designated as *Paenibacillus* by Ashetal [1], and emended by Shida et al. in 1997 [2]. Many of *Paenibacillus* species are known to display plant growth promoting activity, nitrogen fixation, phosphate-solubilizing action and ability to produce extracellular polysaccharides, enzymes, antimicrobials, phytohormones, siderophores. It appears logical therefore that these bacteria and/or their metabolites may find application in medicine, pharmacology, cosmetics, food and feed industry, agriculture, bioremediation technologies, etc. [3].

Earlier bacterial culture PS-K-17 was isolated from wash-offs of wheat grain grown on Belarussian fields. Following phylogenetic analysis of 16S rRNA gene nucleotide sequences (access code MF443394 inGenBank) the isolate was identified as *Paenibacillus* sp. PS-K-17.

The aim of this study: investigation of cultural-morphological and physiological-biochemical characteristics of the strain to estimate its biotechnological potential.

It was found that strain PS-K-17 is Gram-positive, catalase-positive, facultatively anaerobic, rod-shaped and motile (due to peritrichous flagella) bacterium forming ellipsoidal endospores. After 3 days of growth on Sabouraud agar medium the strain produced slimy beige colonies of irregular circular shape of 7–9 mm in diameter, gradually acquiring beige-rosy pigmentation, convex, smooth, and even-edged with viscous texture. After 2 days of growth on peptone-yeast agar with lactose (10 %, w/w) it formed colonies of 5–7 mm in diameter, convex, depressed in the center, viscous, smooth, of beige to beige-rosy color, with even margins.

The strain *Paenibacillus* sp. PS-K-17 neither peptonizes milk nor liquefies gelatin. It assimilates glucose, fructose, galactose, mannose, mannitol, lactose, maltose, sucrose, glycerol, pectin. It is capable to synthesize on agar media with specific substrates and in submerged culture the enzymes beta-galactosidase, amylase, protease, pectinase, cellulose, as well as extracellular polysaccharides and probably carotenoids.

It does not possess antagonistic activity against yeast-like fungi *Cryptococcus flavescens, Saccharomyces cerevisiae, Rhodotorula* sp. – key components of microbial feed products currently developed at the Institute of Microbiology, NAS Belarus.

The data presented above indicate attractive prospects for the use of bacterial strain *Paenibacillus* sp. PS-K-17 as a source of polysaccharides and enzymes hydrolyzing plant polymers. Another plausible application is a constituent of a complex microbial consortium making up an active principle of a novel multifunctional feed additive.

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SCREENING OF PLANT POLYSACCHARIDE- AND LIPID-DEGRADING ENZYMES PRODUCED BY YEASTS FROM COLLECTION FUND

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Bioactive feed additives inhibiting growth of pathogenic and opportunistic microorganisms, proliferating development of intestinal bifido- and lactobacteria, stimulating gut peristalsis, promoting assimilation of macro- and microelements, activating specific and non-specific protective systems, displaying absorbing and other beneficial properties, have been actively introduced into farm stock rations in recent years. Research and development is under way to produce new feed supplements based on live yeast cultures or combinations thereof with pro/prebiotics, enzymes, and other bioactive substances. The investigations are going on to evaluate functional performance of yeast components of feed formulas, including enzymatic, antagonistic, probiotic activities and the role in rumen microbial cenosis [1-4].

The aim of this study – screening of 40 collection cultures of yeast-like fungi from genera *Rhodotorula, Saccharomyces, Torulaspora, Rodosporidium,* for the ability to produce enzymes involved in plant polysaccharide and lipid biodegradation.

According to the obtained data, all examined yeast strains synthesized lipase.

Pectolytic activity was found in 85% of tested variants – in all representatives of *Rhodotorula* genus, in 17 (74 %) out of 23 *Saccharomycetes,* and in *Torulaspora delbrueckii* BIM Y-38 and *Rodosporidium kratochvilovae* BIM Y-157 strains.

Protease production was detected in 23 strains (57 %), alginase and beta-glucanase synthesis – in 6 (15 %) and 7 (17.5 %) yeast cultures, respectively, while beta-galactosidase activity was revealed only in *Saccharomyces cerevisiae* BIM Y-125 strain.

With respect to specific enzyme synthesis, the highest activity was shown by *Saccharomyces cerevisiae* BIM Y-125 (amylase), *Rhodotorula glutinis* BIM Y-159 (protease, pectinase, alginase), *R. glutinis* BIM Y-33 (protease, lipase), *R. glutinis* BIM Y-138, *R. lactosa* BIM Y-118 and *R. mucilaginosa* BIM Y-162 (lipase), *R. lactosa* BIM Y-113 (beta-glucanase).

The most plentiful spectrum of generated enzyme complex comprising 5 constituents was recorded for strains *Rhodotorula glutinis* BIM Y-159 (protease, pectinase, lipase, alginase, and beta-glucanase) and *Saccharomyces cerevisiae* BIM Y-125 (amylase, protease, pectinase, lipase, beta-galactosidase). Strain *Rhodotorula minuta* BIM Y-113 was distinguished by biogenesis of 4 enzymes (protease, pectinase, lipase, and beta-glucanase). The remaining strains were able to produce 1 to 3 of 8 enzymes.

Strains *Saccharomyces cerevisiae* BIM Y-125 and *Rhodotorula glutinis* BIM Y-159 were chosen for further studies aimed at elaboration of biotechnology for production of a complex bioactive feed additive.

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ACTYNOBACTERIA OF THE GENUS *STREPTOMYCES* AND ITS METABOLITES IN BIOREGULATION OF PLANTS

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Actinomycetes are the source of biologically active substances with various chemical structure and spectrum of action. They are filamentous soil bacteria, representing the largest taxonomic unit. Actinomycetes account from 20 to 46% of the total diversity of soil microflora.

The members of the genus *Streptomyces* are active producers of metabolites for biocontrol of phytopathogens population. They are promising objects of biotechnology, as producers of antibiotics of different chemical nature and a wide range of other biologically active substances.

As a result of extensive screening of the collection strains and new isolates of soil streptomycetes, two strains actives against phytopathogenic microorganisms and phytonematodes were selected. Two antifungal compounds of polyene nature of *Streptomyces netropsis* IMV Ac-5025 antibiotic complex have been isolated and identified. One of them is the heptayen antibiotic AC1O5FWR (candidin) and the second is a new unknown tetraene antibiotic. In the antibiotic complex of *S. violaceus* IMV Ac-5027 two compounds has been isolated and identified as anthracycline antibiotics by rhodilunantsin A and rhodilunantsin B. The antinematode activity of its compounds was shown for the first time.

It was established that *S. netropsis* IMV Ac-5025 and *S. violaceus* IMV Ac-5027 synthesize a complex of biologically active substances among which 17 free amino acids (up to 2898 μg/g of ADB), lipids (up to 220 mg/g of ADB) including free fatty acids, phospholipids (over 18%), mono- and diglycerides, triglycerides (over 50%), sterols (cholesterol, ergosterol, sitosterol, stigmasterol, 24-epibrassinolide from squalene precursor), sterol esters, waxes, chitinase and chitosanase enzymes, phytohormone-stimulants, such as auxins (up to 114 μg/g of ADB), cytokinins (up to 106 μg/g of ADB), gibberellins (up to 13 μg/g of ADA), and insignificant amounts of antystress hormone – abscisic acid were determined. An important role of the above biologically active substances on plant bioregulation and on the plant-microbe and microbe-microbe interactions was shown.

The strategy of creation of new multicomponent polyfunctional bioformulations with phytoprotective, growth-stimulating and adaptogenic properties in one biotechnological process have been developed. On the basis of the streptomycetes metabolites complex, the Phytovit (the strain-producer is *S. netropsis* IMV Ac-5025), Violar (the strain-producer is *S. violaceus* IMV Ac-5027) and Avercom nova (the strain-producer is *S. avermitilis* IMV Ac-5015 with chitosan) bioformulations were proposed.

At the molecular and cellular levels were identified the pathways of bioregulatory action of new bioformulations on silencing activity, phenylpropanoid and sterol synthesis, which results in increased plant resistance to biotic and abiotic stresses (priming effect). At the plant organism level the bioregulatory effect of metabolic bioformulations is expressed trough the ability to change the hormonal balance, to stimulate the growth and development of plant, to increase their productivity, to reduce the plant damage and improve the quality of crop production.

Thus, the system approach to the study of complex metabolites of soil strepomycetes was theoretically substantiated and experimentally demonstrated. It was determined the biological (phytoprotective, growth-stimulating, adaptogenic) activity of bioformulations in bioregulation of plants at the molecular, cellular, and organism levels. This gives backgrounds for the creation of fundamentally new multicomponent biological products for the ecologically oriented phytosanitary optimization of agrocenosis.

APPRECIATION OF INOCULATION INFLUENCE ON THE COMPONENTS OF LIPID-PIGMENTS COMPLEX OF WINTER WHEAT UNDER STRESS IMPACT OF HEAVY METALS

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Heavy metals (HM) are most prevalent pollutants of various environmental components, including soils. In food chains, NM alter considerably the intensity of plant metabolic processes (in particular photosynthesis), reducing their productivity and crop quality. It is known, that microorganisms, as mediators between soil conditions and plants, can increase significantly the resistance of macrosymbiont to stress. In this regard, an integrated approach is need to the study of microbial-plant associations. The investigation of physiological and biochemical parameters of plants, in particular the components of lipid-pigments complex, is an integral index of physiological state of plants and of their adaptive potential.

The aim of our research was to evaluate the influence of microbial preparation Phosphoenterin on the components of lipid-pigment complex of winter wheat in the early stages of plant development under the impact of HM.

Vegetation experiments were conducted in a greenhouse on winter wheat - *Triticum durum* L., 5-fold repetition. The preparation Phosphoenterin was used for the inoculation of seeds prior to sowing (control – no inoculated). Plants have been cultivated for 6 weeks in pots (1000 ml) on southern carbonate chernozem. In each pot were added and thoroughly mixed with soil solutions of HM salts: $Pb(CH_3COO)_2$, $CuSO_4$, K_2CrO_4 (levels of HM pollution 1 and 5 MPC), the control – without HM.

The results of our experiments indicate the negative impact of HM on components of lipid-pigment complex in leaves of winter wheat in early stages of ontogenesis. The pollution of soil by HM at 1 MPC reduced the content of chlorophylls a and b in leaves by 2.6 and 1.7 times (to 0.0719 and 0.0408 mg/g, respectively) in comparison with the control, and at 5 MPC – by 4 and 3.5 times (to 0.0468 and 0.0205 mg/g, respectively). The negative influence of HM on the synthesis of carotenoids in leaves of wheat also have been revealed – their content decreased more than 2 times compared to the control (0.0250 mg/g against 0.0588 mg/g). The positive influence of pre-sowing inoculation was established on the content of chlorophylls in the leaves of plants grown on HM contaminated soil: chlorophyll a - by 21% (1 MPC) and 8% (5 MPC), and chlorophyll b - by 32% (1 MPC) and 4% (5 MPC) in comparison with the control. The total content of chlorophylls (a+b) in the leaves of inoculated plants exceeded the control by 25% (1 MPC) and 7% (5 MPC), carotenoids – by 44% (1 MPC) and 5% (5 MPC). The membrane structure of plastids contains the sulfolipids, which support the optimal level of photosynthetic processes in chloroplasts. The negative impact of HM on this component of lipid complex in leaves has been established. Thus, soil contamination of HM at 1 and 5 MPC reduced the content of sulfolipids in leaves by 16% and 31% respectively, compared to control. In leaves of inoculated plants their content increased by 12% (1 MPC) and 5% (5 MPC) in comparison with control. The negative influence of HM on the functioning of the lipid-pigments complex of winter wheat reduced the productivity and height of plants: their mass decreased by 10% and 51% (1 and 5 MPC respectively), height (5 MPC of HM) – by 30%. Inoculation contributed to reduction of stress effects of HM: theirs negative influence on plants was no revealed at the level of 1 MPC contamination of soil; and at 5 MPC – the mass and height of inoculated plants decreased only by 29% and 10%, respetively.

Thus, the positive effect of pre-sowing inoculation of seeds (Phosphoenterin) on the components of lipid-pigments complex in leaves of winter wheat in early stages of plant ontogenesis was revealed, which improved their adaptive potential to HM stress impact.

BIOLOGICAL CONTROL POTENTIAL OF ENTOMOPATHOGENIC FUNGI ISOLATED FROM NATURAL OUTBREAKS IN ROMANIA

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Knowing the natural incidence of entomopathogenic fungi is a stage that could ensure the successful use of these species in biological control. The biological properties responsible for the efficacy of fungal applications usually depend on the origin of the strains (the host insect and the habitat from which they were isolated) as well as interference in the ecosystem.

In Romania, *Beauveria bassiana* was isolated from natural outbreaks with a highest frequency, respectively 55.5%, from species belonging to Order Coleoptera: from adults of Chrysomelidae, Curculionidae and Coccinellidae families and from larvae and pupae of Scarabaeidae family. Although *B. bassiana* was isolated from Order Lepidoptera only with a frequency of 25%, the diseased phytophagous larvae belonging to Yponomeutid Moths, Arctiidae and Geometridae families indicate the susceptibility of lepidopterans to this fungus. This entomopathogen was also isolated from larvae of Hymenoptera and Homoptera with a frequency of just 2.7% and only from adults of Heteroptera, with a frequency of 5%. The strains isolated demonstrated the ubiquitous nature of *B. bassiana* fungi. Thus, 19.4% of *B.bassiana* strains were isolated from soil samples which were collected from forests and parks, 5.5% from decomposed plant detritus in forests, 44.4% from insects collected from agricultural crops and 2.7% from grain storage. The isolation of entomopathogenic fungi from soil evidences the conidia's ability to survive in this habitat and saprophytic ability of the fungus. We have also observed "collaboration" between entomopathogens: entomopathogenic fungi of the genus *Fusarium* sp. and *Lecanicillium* sp., isolated from natural outbreaks, have maintained a chronic pathological condition in a *Calliptamusitalicus* population where *B. bassiana* caused epizootics in the end. The isolation of entomopathogenic fungi from insects also proves a very interesting thing, given the very different conditions of temperature and humidity of the geographical areas from which the host insects were collected: the adherence of fungal spores to host insect cuticle is dependent on its morphological and biochemical properties but germination of the fungus is dependent on the microclimate conditions of the cuticle.

Subsequently, these strains were characterized in the laboratory from biotechnological point of view. The selected strains have been used in research projects in order to control key pests in Romanian agriculture. Demonstration of their efficacy led to their registration at State Office for Inventions and Trademarks (OSIM): Strain BbS1.07 (Patent OSIM/2012) is the strain used for a bioinsecticide for Colorado beetle control, under the name BioProSol and it is also deposited at National Collection of Agricultural and Industrial Microorganisms from Hungary (NCAIM); Strain BbgMm1a/09 (Patent OSIM/2014) – "active substance" for experimental bioinsecticide BioMelCon for *Melolontha melolontha* control; Strain BbIt used in research projects for *Ips typographus* control. Other strains of *B. bassiana* and *Verticillim (Lecanicillium) lecanii* have been successfully used for the biological protection of Romanian crops between 1985 and 1990 and a strain of *B. bassiana*have been used as a component for a nutrient substrate (Patent OSIM /2013).

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INFLUENCE OF THE STORAGE ON SYNTHETIC MEDIA METHOD ON THE VIRULENCE OF ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA*

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This experiment was designed to assess the influence of successive subcultures on synthetic medium of three strains of *Beauveria bassiana* isolated from different host insects (BbLd2/97 isolated from *Leptinotarsa decemlineata,* BbTv1/87 isolated from *Trialeurodes vaporariorum,* BbCi1\94 isolated from *Calliptamus italicus)*. For preservation, *B. bassiana* strains were grown on Potato Dextrose Agar (PDA) medium and after complete sporulation, the cultures were maintained at 4°C in test tubes closed with cotton plugs and sealed with plastic foil. Each strain was transferred on culture medium 6 times over 18 months. In order to detect the influence of successive subcultures on the virulence, we performed bioassays on *Sitophilus granarius* after each subculturing. Observations on the dynamics of insecticidal activity of fungal strains have shown that each transfer on PDA medium has determined a decrease in percentage of mortality. For BbLd2/97 strain, the mortality rate decreased by 1.3% after the 1st subculture, then by 3.9%, 8.2%, 9.4% and 21.5%, corresponding to subcultures 2-6. This means almost two-fold reduction in virulence of BbLd2/97, which caused 94.5% mortality after the first transfer, respectively 50.2% mortality after the 6th subculture. BbTv1/87 strain, whose initial virulence was 1.5 times lower than BbLd 2/97, caused 62.6% mortality after the 1st subculture and 15.6% after the 6th one, which corresponds to a 4-fold reduction in virulence within 18 months. Corresponding to subcultures 1-6, the following decreases in mortality rates were recorded: 2.6, 4.0, 5.0, 12.4 and 23%, respectively. The virulence of the BbCi1\94 strain was maintained high within 9 months, even at the 3rd subculture, the recorded mortality rates ranging from 89.5-84.8%. A considerable decrease in virulence was registered starting with the 4th subculture (77.2% mortality) to the 6th subculture (48.3% mortality), which corresponds to a 1.8-fold decrease in virulence. Regardless of the initial virulence of *B. bassiana* strains, it was reduced 2-4 times over 18 months, during which they supported 6 subcultures. Based on our results, we appreciate that the significance of this reduction in virulence may vary from one strain to another and, at the same strain, from one insect to another, depending on its susceptibility. Thus, what for some *B. bassiana* strains represents only a decrease in virulence, for others it could mean a total loss of it. It was registered a positive correlation between the starting of the saprophytic phase of the fungal cycle and their virulence.

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CORRELATION OF BACTERIOPLANKTON COMMUNITY WITH MAIN PHYSICO-CHEMICAL PARAMETERS OF THE REUT RIVER

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The numerical ratio of the different functional groups of bacterioplankton was analyzed using the data of scientific monitoring of the Reut River, which was undertaken by the Laboratory of Hydrobiology and Ecotoxicology of the Institute of Zoology in June 2018. The Reut River originates on the Northern Moldavian Plateau, collects its waters over a distance of 286 km and flows into the Dniester River near the Ustia village. One of the problems of small rivers in Moldova is their drying in summer season and interruption of their continuity as a result of human activities. The problem has been aggravated in recent years due to climate change, which causes hydrological droughts, especially in summer, but in 2018 also in the spring. During the monitoring, it was recorded that the riverbed doesn't have continuity of watercourse almost to the city of Balti. Thus, the samples were collected at the stations: Balti (upstream and downstream), Orhei (upstream and downstream) and Ustia.

All hydrobionts participate in the biological self-purification of water bodies; however, the main role belongs to the aquatic microorganisms, the quantitative and qualitative composition of which varies depending on the amount and composition of nutrients and pollutants in the water. To evaluate the functional potential of the Reut River's bacterioplankton, the correlation between the numerical density of its functional groups and certain hydrochemical parameters was analyzed. The following parameters were taken into account: water temperature, pH, dissolved oxygen (DO), biochemical oxygen demand (BOD_5); number (CFU/ml) of saprophytes, ammonifiers, denitrifiers, amylolytic and cellulolytic bacteria, phosphate-dissolving and organic-phosphorus-mineralizing bacteria (OPMB), phenol- and petrol-degrading microorganisms. The results of the hydrochemical analysis of the Balti upstream – Ustia sector of the Reut River are presented in Fig. 1.

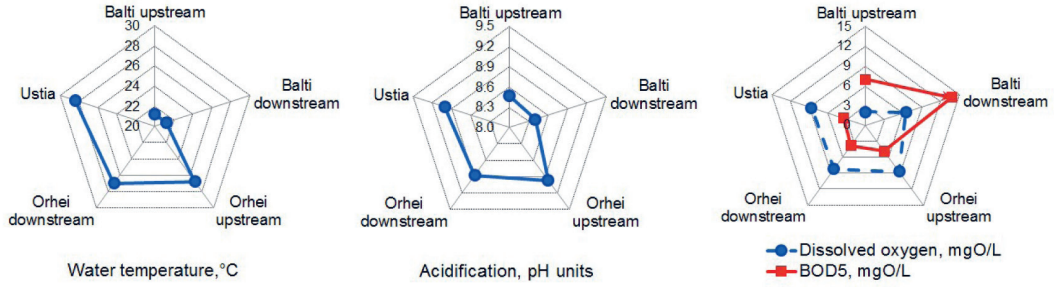

Fig. 1. Temperature, acidification, dissolved oxygen and BOD_5 values of the Reut River's water

To determine the relationship between the number of bacteria in different functional groups and the values of physico-chemical parameters, a correlation analysis was performed (Table 1).

Table 1. Correlation matrix for the Reut River's analyzed parameters

	Temp	pH	O_2 mg/l	BOD_5	Sapr.	PhD MO	PD MO	Ammon.	Denit.	AB	CB	OPMB	Ph-D MO
Temp	1												
pH	0.9862	1											
O_2 mg/l	0.8206	0.7780	1										
BOD_5	-0.8171	-0.8488	-0.3566	1									
Saprophytes	0.4730	0.3259	0.4878	-0.2539	1								
Phenol-degrading MO	-0.7846	-0.7256	-0.4030	0.8318	-0.6740	1							
Petrol-degrading MO	-0.9766	-0.9391	-0.7676	0.7985	-0.5723	0.8718	1						
Ammonifiers	0.1588	0.0069	0.3604	0.1003	0.9100	-0.3187	-0.2292	1					
Denitrifiers	-0.4527	-0.5861	-0.0566	0.7372	0.4588	0.2685	0.3487	0.7051	1				
Amylolitic bacteria	0.2175	0.1008	0.2603	-0.1488	0.8409	-0.4049	-0.2429	0.9081	0.4251	1			
Cellulolitic bacteria	0.1917	0.0586	0.4050	0.0525	0.8560	-0.2675	-0.2166	0.9749	0.6063	0.9488	1		
OPMB	-0.8655	-0.8143	-0.5921	0.8436	-0.7119	0.9064	0.8764	-0.4411	0.2936	-0.5994	-0.4762	1	
Phosphate-dissolving MO	0.6281	0.5024	0.8029	-0.2137	0.8974	-0.5380	-0.6649	0.8298	0.3866	0.7047	0.8169	-0.6752	1

Then, the correlation coefficients between the biotic and abiotic components of the matrix were presented in a graphical form (Fig. 2).

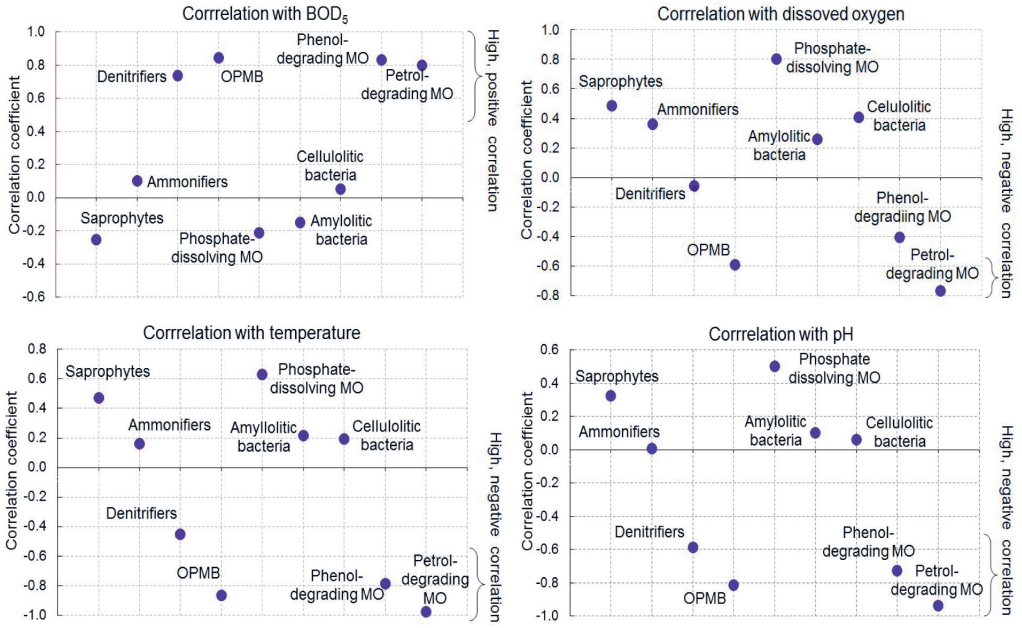

Fig. 2. Graphical interpretation of the correlation coefficients between the biotic and abiotic components of the Reut River's ecosystem

Conclusions:

It was noted that the deficit of watercourse on the Rediu Mare – Balti sector is the reason why the river flow downstream from Balti is mainly formed by urban wastewaters.

Correlation with BOD_5 was high and positive ($r \geq 0.8$) for such groups of bacterioplankton as phenol- and petrol-degrading microorganisms, denitrifiers and organic-phosphorus-mineralizing bacteria. Correlation with dissolved oxygen was high and positive ($r = 0.8$) only for phosphate-dissolving bacteria, while for petrol-degrading microorganisms it was also strong, but negative ($r = -0.77$). Phenol- and petrol-degrading microorganisms, as well as organic-phosphorous-mineralizing bacteria, demonstrated a high and negative correlation with water temperature ($r > -0.9$).

Most of the investigated groups of bacterioplankton showed a negative correlation with alkalinization of the habitat, that is, an increase in pH. Ammonifying bacteria, as well as amylolytic and cellulolytic bacteria, showed a low correlation with all the abiotic factors analyzed. Thus, the content of nutrients and contaminants in the investigated section of the river provides an acceptable habitat for survival and development (in summer season) of such groups of bacterioplankton as denitrifiers, phosphate-dissolving and organic-phosphorus-mineralizing bacteria, as well as phenol- and petrol-degrading microorganisms.

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MICROBIAL CELLULOLYTIC COMPLEX FOR PLANT RESIDUES DECOMPOSITION

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The use of crop residues as organic ameliorant is an efficient, cost-effective and environmentally friendly way aiming at the recovery of soil organic matter reserves, as well as improvement of biological and physical properties of soil. Cellulolytic microorganisms are an important component of soil biocenosis, which ensure plant residue decomposition. Anthropogenic impact on the agrocenoses caused the disturbance in the normal processes of decomposition and remedial measures a needed. The use of microbial preparations is one of the ways to accelerate the crop residues decomposition, as well as to preserve and restore soil fertility. Production of such preparations is an urgent problem of agricultural microbiology and biotechnology.

The aim of the research was to develop a complex biological preparation for effective management of microbiological processes of decomposition cereal crops residues.

Association of microorganisms (CA-5M) with the level of cellulolytic activity of 70% was isolated from the arable layer of southern chernozem (fallow). The growth dynamics of microorganisms forming the association was studied under conditions of periodic cultivation in a liquid nutrient medium with chopped wheat straw as the sole carbon source. An increase in the total bacterial titer of 200 to 600 million colony-forming units (CFU) per ml was noted during the period from 24 to 48 hours of cultivation. Two peaks of titer increase appeared due to spore-forming bacteria: 24 and 72 hours after the beginning of cultivation. The number of micromycetes increased after 48 hours of cultivation. The components of the medium, optimal parameters of cultivation and stabilizing additives were selected to increase and preserve cellulolytic activity of the studied association.

Artificially created associations of microorganisms are used in the microbiological industry to intensify technological processes. Nitrogen-fixing, phosphate-mobilizing, bio-protective and growth-stimulating bacteria of the genera *Alcaligenes, Paenibacillus, Enterobacter, Azotobacter, Agrobacterium* were selected from the collection of microorganisms (https://pm.cytogen.ru/content/passports/krymskaya-kollekciya-mikroorganizmov-fgbun-niish-kryma-kkm-fgbun-niish-kryma) to create the multicomponent complex biological preparation. The increased activity of CA-5M was noted during the co-cultivation with the studied bacterial strains. There was also an increase in resistance to abiotic stress factors (high temperature, moisture deficiency), which is relevant for arid climatic conditions that happen more often in the south of the country.

The treatment of straw and stubble with the complex biopreparation with further incorporation into the soil increases the rate of mineralization twice, reduces the phytotoxicity of the soil by 1.5%, and contributes to an increase by 5% in the yield of the next crop, such as winter wheat. The use of biological preparations for green-manured fallow (winter triticale) promotes an increase in the biological activity of the soil and improvement of the content of organic matter.

Thus, it has been shown that cellulolytic associations of microorganisms are the promising targets for biotechnology. A five-component microbiological complex for the plant residue decomposition was developed.

PRODUCTION OF EXTRACELLULAR PHYTOHORMONES GIBBERELLINS BY BIOTECHNOLOGICAL STRAINS OF *BRADYRHIZOBIUM JAPONICUM*

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In the root zone of plants nitrogen-fixing bacteria are able to synthesize a wide range of biologically active compounds, including compounds of phytohormonal nature. We have previously studied the ability of soybean nodule bacteria *Bradyrhizobium japonicum* to synthesize such extracellular phytohormones as auxins, cytokinins and abscisic acid. It is known that phytohormones with growth stimulation activity (gibberellins) have wide spectrum of influence on physiological processes in plants. Gibberellins cause stem elongation in plants, which is based on the cell spreading and increased mitotic activity.

The aim of this work was to study the synthesis of gibberellic extracellular compounds by biotechnological strains of nitrogen-fixing symbiotic bacteria *B. japonicum*.

The objects of research were highly effective biotechnological strains of *B. japonicum*: UCM B-6018, UCM B-6035 (Ukrainian Collection of Microorganisms) and KC19, KC23 (IAMAM NAAS Collection of Microorganisms).

All *B. japonicum* strains were grown in a liquid yeast extract mannitol medium in periodic conditions. Phytohormones with gibberellic activity were isolated with ethyl acetate in the ratio 1:1 (v/v) at pH 2.5. The obtained extracts were evaporated at 40–45 °C. Dry residue was dissolved in ethanol and used for physical and chemical analysis of phytohormones. The total ability to synthesize gibberellin by rhizobia was evaluated in specific phytotest. Qualitative and quantitative determination of gibberellins was performed by high performance liquid chromatography (HPLC) method using a liquid chromatograph Agilent 1200 (*Agilent Technologies, USA*) and mass spectral detector Agilent G1956B. For comparison, standard solutions of gibberellins GA_3, GA_4 and GA_7 (*Sigma-Aldrich, Germany*) were used. The quantity of synthesized gibberellins was expressed in μg per 1 g of absolutely dry biomass (ADB).

By the phytotests results it was shown the presence of gibberellic nature compounds in cultural liquids of all studied soybean nodule bacteria *B. japonicum*.

Results obtained by HPLC have confirmed the phytotest results about presence of extracellular compounds with gibberellic activity in all rhizobia strains. Among active biotechnological *B. japonicum* strains the largest amount of gibberellins was synthesized by strains *B. japonicum* UCM B-6018 (161.1 μg/g ADB of GA_3 and 5.1 μg/g ADB of GA_4) and *B. japonicum* KC23 (201.2 μg/g ADB of GA_3 and 4.3 μg/g ADB of GA_4). Slightly less amount of gibberellins was synthesized by another active strain *B. japonicum* UCM B-6035 – 136.7 and 5.3 μg/g ADB respectively. In the supernatant of *B. japonicum* strain KC19 was detected GA_3 (85.0 μg/g ADB) but not detected GA_4. Also trace amounts of GA_7 were found in all extracts. It should be noted, that all the strains produced much bigger amount of GA_3 than GA_4 and GA_7. It is GA_3 that has the highest biological activity in plants among gibberellic compounds.

Thereby obtained data prove the ability of soybean Bradyrhizobia to synthesize phytohormones gibberellins. Besides there is reasons to say about possible perspectives of using effective strains for stimulation seeds germination and raising the productivity of soybean plants as preparations not only with nitrogen fixing activity, but also with stimulation of gibberellin production activity.

MICROBICOCENOSIS OF *TRITICUM AESTIVUM* L. RHIZOSPHERE DEPENDING ON VARIETY AND GROWING CONDITIONS

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Common wheat (*Triticum aestivum*) occupies the leading position in the world production of grain crops. It is capable to grow in a wide range of soil and climatic conditions. The microbial surrounding is important for implementation of the plant productive potential. Microorganisms are able to provide plants with mineral nutrition elements, to stimulate growth and development and increase their immune status. The study of the quantitative composition of the microbial communities of the soil makes it possible to determine the direction of the processes in the transformation of organic matter, which is an important basis for increasing the productivity of plants and the fertility of soils.

The present study evaluates the number of microorganisms in the rhizosphere of two varieties (Ermak and Lydia) of common wheat, grown in different soil and climatic conditions (southern chernozem of the steppe and foothill zone of Crimea and typically chernozem of the Rostov region).

The number of soil microorganisms (expressed in in colony-forming units (CFU) per g of completely dry soil) of the main ecological and trophic groups (ammonifying and amylolytic bacteria, nitrogen fixing, micromycetes, cellulosolytics, streptomycetes, oligotrophs and pedotrophs) of the wheat rhizosphere, was determined by generally accepted methods. The study of the rhizosphere microbiome structure was performed using high-throughput DNA sequencing of 16S rRNA gene libraries.

For the aboriginal microbiota of the wheat rhizosphere, the conditions of chernozem of southern steppe zone were more favorable than in the foothill zone. This is evidenced by the 3.2 times increasing of the number of oligotrophs in the rhizosphere of the Lydia variety (0.6 ± 0.1 million CFU / g of soil) and by 2.6 times of the number of pedotrophs (3.3 ± 0.2 million CFU / g of soil). The number of microorganisms, that transform organic and mineral nitrogen compounds in the rhizosphere of the Lydia variety, grown in the steppe samples by 2.3 times and in the foothill zone samples – by 1.8 times (the amount was 3.8 ± 0.3 and 5.1 ± 0.5 million CFU / g soil, respectively). The number of majority studied groups microorganisms in the rhizosphere of the Ermak variety was higher than Lydia under conditions of both zones of southern chernozem and typically chernozem. The quantity of the ecological-trophic groups of microorganisms in the *T. aestivum* rhizosphere samples from the foothill zone of southern chernozem and typically chernozem are more closely related.

According to the data of 16S rRNA gene libraries pyrosequencing, the ratio of the taxa of microorganisms was different in sundries zones of wheat cultivation. Its varieties also have an effect on the distribution of the shares of individual microbial groups.

Thus, were established changes in number of microorganisms of the main ecological-trophic and systematic groups of *T. aestivum* rhizosphere of the southern and typical chernozems, which depend on the reaction of the variety to the soil-climatic conditions of cultivation.

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NEUTRALIZATION OF WHITE PHOSPHORUS BY CULTURE *ASPERGILLUS NIGER*

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White phosphorus P_4 is one of the most dangerous pollutants of the environment. Nevertheless, it is used in industry and for military purposes, so it cannot be ruled out that this substance is exposed to the environment. Consequently, methods of detoxifying P_4 are needed, including biological means.

For the first time, we have successfully cultured microbes (mold fungus of the genus *Aspergillus*) in a medium containing P_4 as the sole source of phosphorus. In this novel medium, the microorganism grew and did not experience phosphorous starvation (Fig.). That is, the fungus oxidized white phosphorus to phosphate, which is a primary necessity for life! *Aspergillus* grows in a medium with white phosphorus concentration of up to 1%. This exceeds the TLC of P4 in wastewater by about 5000 times! Across the Globe, this is the first example regarding the inclusion of white phosphorus into the biospheric cycle of elemental phosphorus.

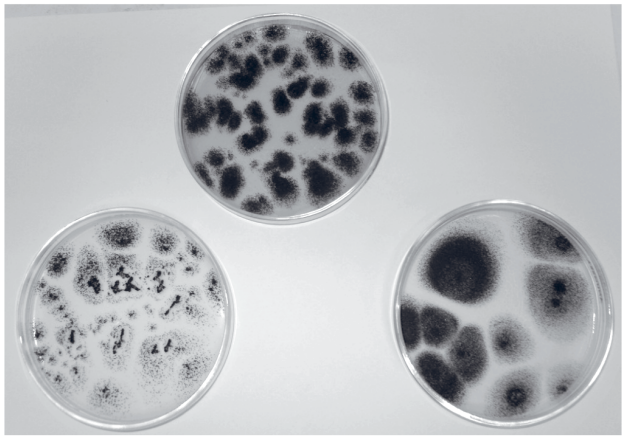

Fig. The first re-inoculation of resistant fungi A. niger. To the left is the medium without a source of phosphorus in it, 33 weakened colonies observed. Above is a medium, containing phosphate: growth of 49 spore-forming colonies of A. niger was observed. On the right is a medium with 0.05% white phosphorus: there were 11 large spore-forming colonies of A. niger. Photographs were taken six days after the inoculation.

We identified this microorganism as a new strain of *A. niger*. The strain was designated as *A. niger* AM1. The nucleotide sequence of the strain is published in the GenBank database, where it is assigned the number KT805426.

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SPECIFIC FEATURES OF THE DIGESTION AND THE METABOLISM OF BIRDS

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Compared to other species of farm animals, the chicken is characterized by high intensity of exchange processes, high absorption capacity and energy efficiency of feed promoting precocity and high productivity. It has a body temperature higher than that of mammals (40-42 °C), more oxygen consumption per unit of live weight, rapid breathing and pulse. Therefore, in order to maintain life, high metabolism and productivity of the bird needs a sufficient amount of energy and a complex of nutrients. The structure and functioning of the digestive system in birds have their own characteristics throughout its length, from the oral cavity to the cloaca.

Organs of digestion in poultry include: oral cavity, pharynx, upper esophagus, goiter, lower esophagus, glandular and muscular stomachs, small intestine, caeca, rectum and cloaca, as well as the digestive glands pancreas and liver. The peculiarities of the structure and functioning of the digestive system include the absence of teeth; food is grasped by the beak and is swallowed whole, as well as the presence of the oropharynx.

Food enters the relatively long esophagus into the crop, where it is exposed to enzymes and microflora. The volume of crop and its storage capacity depends on the live weight of the bird. With the help of peristaltic crop contractions, the food is mixed and fed in portions to the glandular and then into the muscular stomach as they are released. The acidic environment in the stomachs contributes to the action of pepsin, which breaks down easily digestible proteins to polypeptides. More intensive digestion occurs in the muscular stomach and it is the intensive increase in the muscular stomach that ensures the process of adapting chickens to solid food nutrition. The intestine in the bird is relatively short in comparison with mammals. In chickens, the length of the intestine is 165-230 cm, which is 5-6 times the length of the body.

A significant part of the nutrients – proteins, fats, carbohydrates – is digested in the duodenum with the participation of bile, pancreatic juice and intestinal glands. Bile of birds differs from the bile of other animals by the presence of stearic acid.

In the lower parts of the small intestine, the cleavage of nutrients is completed with the help of enzymes of intestinal juice and the absorption of the bulk of digestion products occurs. However, the cleavage of cellulose is 10-30%, since enzymes that promote the digestion of cellulose – cellulases, hemicellulases, peptidases, are not synthesized in the digestive tract of birds.

According to the norms, the optimal fiber content in broiler chicken rations is 3-4%, and the maximum 5%. Increased nutrient intake will contribute to the fact that indigestible residues will be the basis for excess microbial growth in the intestine. At the same time, a lack of fiber leads to a violation of digestion, and as a consequence, reduced bird productivity, diseases and death are possible.

The digestible nutrients of mixed fodders are used to build organs and tissues, as well as a source of energy, which contributes to the rapid growth of young animals. However, reserves of nutrients in birds are limited and the of fodder, the lack of vitamins and minerals in them negatively affects its productivity and health. Therefore, high productivity can only be achieved from a healthy bird.

BIOLOGICAL PROPERTIES OF STRAINS AND COMPLEX BIOFORMULATION ECOPHOSFORYN EFFICIENCY

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Among the modern agrotechnologies aimed at increasing agricultural plants productivity and crop quality, complex biopreparations based on agronomically useful microorganism associations and their metabolites take the significant place. These bioformulations make it possible to obtain ecologically friendly production and they are an integral part of organic farming.

Bacterial bioformulation Ecophosphoryn for cereals, technical, vegetable and other crops has been developed at the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Science of Ukraine. It has received the Quality Certificate "Organic Standard" for use in organic agriculture (according to the IACB Equivalent European Union Organic Production and Processing Standard to the Regulations EU No. 834/2007 and No. 889/2008).

Ecophosphoryn components are strains of soil phosphate-mobilizing and nitrogen-fixing bacteria with a wide range of agronomically useful properties: the ability to mobilize insoluble organophosphates, fix the atmospheric nitrogen, synthesize a complex of biologically active substances, including exopolysaccharides, phytohormones, and antibiotic compounds for the phytopathogens biocontrol. Strains-bioagents also have a urease activity, ability to bioremediate radionuclide contaminated soils, and a resistance to chemical fungicides. It allows using Ecophosphoryn in integrated plant protection technologies.

Ecophosphoryn phytostimulating and biocontrolling activity has been shown in laboratory, vegetative and field experiments on buckwheat, barley, wheat, maize, sunflower, flax, cabbage, tomato and other crops.

Under the inoculation of the seeds of Podolianka cultivar winter wheat with Ecophosforyn, a stimulation of development of a photosynthetic apparatus has been observed. The leaf area on one plant, the accumulation of chlorophyll in leaves and the productivity of photosynthetic activity of a leaf surface unit have increased by 20%, 40% and more than 30%, respectively. The harvest of grain has increased by 25%, wherein the quality of grain has improved (weight of thousand grains and grain nature). The pre-sowing seeds treatment with Ecophosphoryn has contributed to the increase in the grain yield of Rannia 93 cultivar spring wheat by 10-19% (3-6 c/ha). Ecophosphoryn used during the sunflower growing season has contributed to the increase in yield by 300 kg/ha.

Ecophosphoryn joint use with Maxim XL 035 FS fungicide (with 0.1 application rate) in the arid vegetation period conditions has effectively protected the crops from fusarial-helminthosporous root decay and contributed to a significant increase in the yield of Myronivska 65 cultivar winter wheat by 2.6 c/ha. High efficiency has been shown by Ecophosphoryn in a combination with fungicide Acrobat MC with a reduced by 25% rate of consumption on tomatoes of Flora cultivar. The average efficiency against tomato alternaria was 72.9% compared to 73.4% in a variant with a fungicide with a full rate of consumption, wherein the yield by 15% has increased.

Thus, thanks to the multi-vector beneficial effect on plants, Ecophosphoryn helps to improve their nutrition, increase their immune status and stress resistance, control phytopathogens, activate development of microbial-plant systems, increase productivity and crop quality.

RHIZOSPHERE MICROBIOME DIVERSITY AND SOYBEAN PRODUCTIVITY UNDER COMPLEX INOCULATION

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At present, soybean is in a great demand as a legume. In view of that, its cultivated areas are growing in Ukraine and all over the world. To improve its productivity, advance the crop quality and reduce doses of nitrogen fertilizers, bioformulations based on nodule nitrogen-fixing bacteria are applied. No less important role in crop yields increasing and soil fertility stabilizing plays the conservation of microbiota biodiversity in plant rhizosphere.

The complex bioformulation Ecovital based on 3 strains of soybean nodule bacteria (*Bradyrhizobium japonicum* UCM B-6018, UCM B-6023, UCM B-6035) and phosphate-mobilizing bacilli (*Bacillus megaterium* UCM B-5724) has been developed at the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine

To study the effect of complex inoculation on the rhizosphere microbiocenosis of the Annushka cultivar soybean the soil total microbial DNA of the root zone was analyzed by using the high-performance pyrosequencing method. Before sowing the seeds were inoculated with the complex microbial preparation Ecovital, and in the control variant they were treated with the sterile water.

In the soybean rhizosphere microbiome, 12 phylums of bacteria were identified: *Acidobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, Planctomycetes, Proteobacteria, Verrucomicrobia* and *WPS-2*. The dominant were the phylums of *Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria* and *Acidobacteria*. Their relative quota in the microbiome increased (except for phylum *Actinobacteria)* in the variant with using the complex inoculum.

The bacteria capable of active roots colonization and joining a phytopartner in symbiotic relationship play an important role in legume-rhizobial symbiosis. Rhizobia and PGPB (Plant Growth Promoting Bacteria) are often found among the representatives of *Rhizobiales* and *Bacillales* orders belong to these bacteria.

In the soybean rhizosphere microbiome, we have found 6 families of the *Rhizobiales* order. The representation of *Bradyrhizobiaceae, Rhizobiaceae, Phyllobacteriaceae, Methylobacteriaceae, Hyphomicrobiaceae* families was increased in the rhizosphere soybean microbiome in a variant with the inoculant. Bacteria of the *Xanthobacteraceae* family were detected in the variant with Ecovital only. The uncultivated forms of the *Bosea* genus of the *Bradyrhizobiaceae* family were identified in the same variant.

Five families were detected from the *Bacilli* class of *Bacillales* order. The *Bacillaceae, Paenibacillaceae* and *Planococcaceae* families were dominant. The representation of bacteria of the *Bacillaceae* and *Paenibacillaceae* families increased in the variant with Ecovital. The relative quota of representatives of the *Planococcaceae* family decreased from 1.3% in the control variant to 0.9% in the variant with Ecovital. The *Sporolactobacillaceae* and *Thermoactinomycetaceae* families were detected only in the variant with bacterization.

Inoculation of soybean seeds with Ecovital promoted the increase of plant productivity from 17.6 c/ha in the control variant to 19.4 c/ha in the variant with the bioformulation.

Thus, inoculation of soybean seeds with the complex microbial formulation Ecovital contributed biodiversity growth of the *Alfaproteobacteria* class of the *Rhizobiales* order and the *Bacilli* class of the *Bacillales* order representatives in the rhizosphere microbiome, as well as, plant productivity increase.

White and Gold

Biotechnology

Gene-based Bioindustries

Bioinformatics

Nanobiotechnology

ISOLATION OF TRIFLURALIN DEGRADING MICROBIAL CONSORTIUM

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The genetic heterogeneity of microbial communities from pesticide-contaminated soil allows some microorganisms to adapt to changes in the characteristics of the energy substrate. The biochemical and genetic basis of microbial transformation of xenobiotics was linked to several genes/enzymes providing microorganism with the ability to degrade halogenated organic pesticides. Despite the high speed of reproduction of the microorganisms, the process of adaptation in the soil takes decades. Therefore, the most effective way of searching for the microorganism-destructor of xenobiotics is to isolate it from the natural environment and to adapt the microorganism to high concentrations of the toxicant. Due to the high toxicity of halogenated pesticides and, often, the presence of two or more contaminants in polluted soil, it is necessary to search for ways to optimize the biooxidation processes of such substrates.

In this research, a technological scheme was developed for the process of isolation a microbial consortium involved in the degradation of herbicide trifluralin, using iron (II, III) oxide (Fe_3O_4 or magnetite) nanoparticles and zero-valent iron Fe(0) nanoparticles.

The consortium of microorganisms was created on the basis of an accumulative culture obtained by inoculation of soil contaminated with pesticides into the PAS nutrient medium, pH varied from 5.0 to 6.5. The evolution of microbial consortium was observed through series of passages. Regulating the acidity of medium for accumulation culture, it is possible to control the development of a microbial consortium to the predominance of bacterial or fungal cultures. Depending on the acidity of the medium, trifluralin promoted an increase in the number of bacteria and a sharp increase in the number of micromycetes.

A consequent increase in the concentration of trifluralin in the medium for accumulation culture has led to a reduction (depletion) in the species diversity of bacteria and micromycetes. An addition of Fe_3O_4 and Fe(0) nanoparticles to the medium restored the species diversity of these microorganisms.

Every increase in the trifluralin concentration in the medium with acidity of pH 5.0 led to a gradual decrease in the number of bacteria, while the additon of Fe(0) nanoparticles in the same condition contributed to an increase in their numbers. The magnetite and zero-valent iron nanoparticles stimulated the growth and reproduction of micromycetes on the initial etapes of development of the consortium, when trifluralin concentration range was 100-200 mg/L. Then, when the concentration of trifluralin reached to 300-400 mg/L, the number of micromycetes did not depend on the acidity of medium, or on the presence of nanoparticles.

NANOMETHODS FOR OBTAINING LIPOLYTIC ENZYME PREPARATIONS FROM FUNGI

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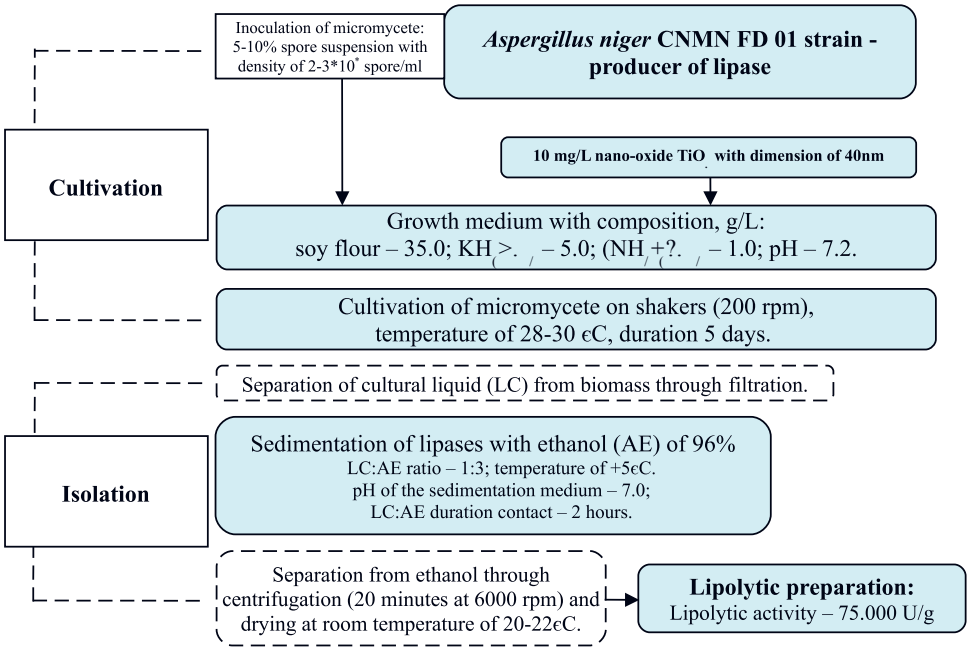
Lipolytic enzymes have important applications in various fields of industry and medicine. Lipases are used to process food, leather, textile fibers, detergents, paper, fine chemicals, pharmaceuticals, cosmetics, biofuels etc. The major lipase producers are fungi from genera *Aspergillus, Penicillium, Rhizopus, Candida, Mucor*. An innovative concept in the biotechnological production of microbial lipases is the use of nanoparticles as stimulating and regulating factors of biosynthesis. The nanoparticles present a unique tool in manipulation of the biosynthetic activity of microorganisms with proven efficiency on biotechnological objects from different taxonomic groups. The aim of this research was to develop methods for obtaining new lipolytic preparations with the application of nanoparticles as factor of influence.

The object of the study was *Aspergillus niger* CNMN FD 01 fungi strain with biotechnological significance, with high and stable synthesis of exocellular lipase (MD 2362). The culture was stored in the National Collection of Nonpathogenic Microorganisms of the Institute of Microbiology and Biotechnology, Moldova. In order to increase the biosynthetic capacity of *A. niger*, the influence of TiO_2 (of 20nm, 40nm, 1μm) and Fe_3O_4 (of 10nm, 30-35nm, 70nm) metal oxides were tested. Nanoparticles were included into culture medium in the following concentrations: 1.0; 5.0; 10.0 and 15.0 mg/L.

In conclusion, we developed an integrated scheme for obtaining new lipolytic enzyme preparation with a 10x degree of purity, having a lipolytic activity of 75.000 U/g and a specific activity of 2205 U/mg protein (Figure).



It included the cultivation of *Aspergillus niger* CNMN FD 01 strain in the presence of 10mg/L TiO_2 nano-oxide with dimension of 40 nm, followed by lipase separation through sedimentation with 96% ethanol.

Obtained lipolytic preparation can be used in zootechny, food industry, light industry and bioremediation processes.

EFFECT OF ZNO NANOPARTICLES ON BIOMASS PRODUCTION AND CARBOHYDRATES OF *SACCHAROMYCES CEREVISIAE* CNMN-Y-20 STRAIN

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Today, there is a debate between scientists on the toxicity of ZnO nanoparticles, which are often used in food, cosmetological and pharmaceutical industries. Some researches show that only nanoparticles smaller than 30 nm are toxic, other researches showed that nanoparticles are toxic to living organisms regardless their size.

It is known that carbohydrates perform a number of functions in the cell. Thus, they serve as reserve substances, participate in the transport of nutrients, ensure the correct structure and protection of cell membranes and, in complex with other substances, regulate the synthesis of different substances, cell growth and its multiplication. Stimulation of carbohydrate biosynthesis usually occurs in response to various unfavorable cultivation conditions, such as suboptimal temperatures, high osmotic pressure, deficiency in nutritional sources, presence of various harmful substances in the nutrient medium, and indicates to a probable stress state of the microbial cells.

In this context, the aim of the research was to evaluate the effect of ZnO nanoparticles with <100 nm size on biomass production and carbohydrate content of *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain, a β-glucans active producer, deposited in the National Collection of Nonpathogenic Microorganisms.

There were used ZnO nanoparticles in powder form ALDRICH, dispersed in poly(N-vinylpyrrolidon) in concentrations of 0,5; 1; 5; 10 and 15 mg/L. The nanoparticles were added as an emulsion in YPD culture medium at the time of inoculation with the seed material. As a control was examined variant without nanoparticles. The yeast biomass was collected by centrifugation after 120 hours of cultivation. Total carbohydrate content was also determined after 120 hours.

The obtained results demonstrate that biomass amount presented a relative stability for the yeast strain cultivated in the presence of <100 nm ZnO nanoparticles (Figure). Its content, calculated on 1L culture medium, varied practically at the control level.

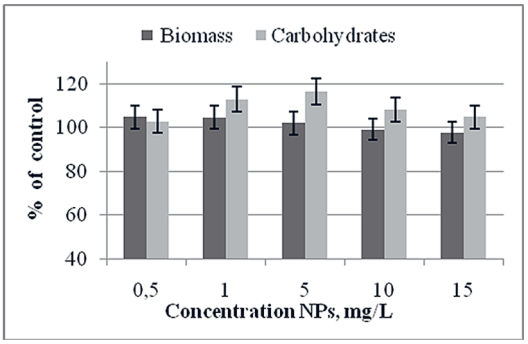

Figure. ZnO nanoparticles effects (<100 nm) on biomass production and total carbohydrate content in *S. cerevisiae* CNMN-Y-20 strain.

The influence of ZnO nanoparticles (<100 nm) on total carbohydrate content has revealed different values depending on the concentration of used nanoparticles. The results showed an increasing tendency of their content in the presence of nanoparticles in 1-15 mg/L concentrations. Thus, carbohydrate content was up to 16.5% higher than control (Figure).

Therefore, the results denote that the effect of ZnO nanoparticles (<100 nm) depends on their concentration in culture medium. Namely, ZnO nanoparticles in concentrations of 1-5 mg/L have been manifested as stimulators of carbohydrate biosynthesis in *S. cerevisiae* CNMN-Y-20 strain.

EFFECT OF TRIFLURALIN AND IRON NANOPARTICLES ON THE CULTURAL PROPERTIES AND GROWTH OF STREPTOMYCETES

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The problem of pesticide degradation in soil is a major current issue of environmental ecology. The most effective destroyers of xenobiotics are microorganisms. The ability of microorganisms to destroy pesticides is related to long-term adaptation to environmental conditions and their activity in many biochemical processes. Numerous studies have shown that greater biodegradation efficiency of organic pesticides takes place under the action of bacterial strains of the genus *Pseudomonas*, *Bacillus*, or fungi of the genus *Trichoderma*, *Penicillium*, *Cladosporium*, *Aspergillus,* and *Fusarium*. Among the non-pathogenic actinobacteria the genus *Rhodococcus* are a unique group; they exhibit specific survival skills in critical conditions. They possess the ability to assimilate complex chemical substrates, such as petroleum hydrocarbons, phenolic compounds, humus substances, waxes, resin acids, etc. One important characteristic is their ability to grow and develop in a wide range of concentrations of chemical compounds, which allows surviving in aquatic basins, soils and other polluted areas. There are no available results in this regard on streptomycete strains.

Based on the above, the aim of the research was to study the ability of nanoparticles to stimulate the growth of streptomycetes in the presence of trifluralin. Two streptomycetes strains from the National Collection of Non-pathogenic Microorganisms were used in the study: *Streptomyces sp.* 205 - isolated from soil polluted with pesticides and *Streptomyces sp.* 12 - isolated from the chernozem of the Central Part of Moldova.

There is sufficient data in the literature on the use of nanobiotechnologies to improve the environmental situation, especially for the detection of the active xenobiotic destructive strains. Nanoparticles have been shown to have both stimulatory and inhibitory effect on biomass productivity and composition of various microorganisms: yeasts, fungi, cyanobacteria, etc.

Experiments on the action of trifluralin (200 and 300 mg/l concentration) in the presence of Fe^0 on the growth of streptomycetes on glucose-free Czapek agar medium demonstrated that the addition of Fe^0 nanoparticles at the concentration of 25 mg/l caused changes in the color of the substrate mycelium of *Streptomyces sp.* 12 from pale yellow to dark red. The appearance of the brown pigment (type 1), the change of chamois color to pale honey and the absence of the 2-type soluble pigment was observed.

The following changes were observed in cultural properties of *Streptomyces sp.* 205 strain: the whitish substrate mycelium became grayish in 1st type colonies; the whitish substrate mycelium became reddish-brown and brownish soluble pigment appeared in 2nd type colonies.

Upon addition of 50 mg/l Fe^0 of nanoparticles in glucose-free Czapek agar medium, for both strains were detected 3 types of colonies differing by insignificant changes of the aerial mycelium and non-significant changes in the color of substrate mycelium. For *Streptomyces sp.* 12 the yellow-pale color is changed to the grayish one, and in other two variants the mycelium of the substrate was red-brown and the brown soluble pigment around the colonies with the white and gray aerial mycelium.

The highest growth activity of the studied strains was observed at the cultivation of *Streptomyces sp.* 12 on Czapek medium + 200 mg/l trifluralin (84.25%), and of *Streptomyces sp.* 205 - on Czapek medium + 200 mg/l trifluralin + 50 mg/l nanoparticles (92.96%). Thus, the conducted studies have shown that the presence of nanoparticles positively influence the growth and development of some strains of soil streptomycetes in the medium with trifluralin.

IMPACT OF MAGNETITE AND ZERO-VALENT IRON NANOPARTICLES ON GROWTH OF STREPTOMYCETES

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Streptomyces is the most widely studied and well-known genus among actinomycetes. Streptomycetes, due to their extracellular enzymes, have a great potential for the biodegradation of organic and inorganic toxic compounds. Several studies have demonstrated the ability of streptomycetes to grow and degrade several chemical families of pesticides, including organochlorine, organophosphorous, polyaromatic hydrocarbons. Last decade streptomycetes are successfully used in the synthesis of metal nanoparticles, especially of silver and gold, less of manganese, zinc and copper nanoparticles.

Among metal-based engineered nanomaterials, iron nanoparticles (NPs) are, probably, the most used for bioremediation of a broad spectrum of pollutants. Iron-based NPs are expected to be nontoxic, due to using Fe atom in several pathways of cell metabolism and, therefore, low iron toxicity. But there is a series of investigations that prove the toxic action of iron NPs on different microorganisms. Regarding the interaction between iron NPs and streptomycetes, there is a little information in specialized literature, but the existing data attests the resistance of streptomycetes to the action of iron NPs.

The present study aims to determine the impact of magnetite NPs and zero-valent iron (ZVI) NPs on growth of *Streptomyces* strains. *Streptomyces* strains were isolated from soil long-term polluted with obsolete pesticides, DDT and trifluralin. Encapsulated NPs were synthesized by chemical co-precipitation method, in the presence of poly-N-vinylpyrrolidone (PVP) used as a stabilizer. Magnetite NPs were prepared using iron (II) sulfate and iron (III) chloride. ZVI NPs were prepared by chemical reduction from ferric chloride solution. The inhibition activity of magnetite and ZVI NPs was evaluated using express-method.

Each streptomycete strain had an individual reaction to the solutions of iron NPs. Sensitivity of the strains varies depending on the chemical form of NPs and the individual peculiarities of each strain. In most cases, both magnetite and ZVI NPs had a stimulating effect on the growth of streptomycetes, ZVI NPs had no inhibitory effect on their growth. Most of the studied streptomycetes were found to be sensitive to trifluralin, fluorinated dinitroaniline herbicide. Mixing the solutions of iron NPs and trifluralin resulted in the reduction of trifluralin toxicity.

SYNTHESIS OF SELENIUM NANOPARTICLES ON FRACTION OF POLISACCHARIDES DERIVED FROM *SPIRULINA PLATENSIS* BIOMASS

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Selenium nanoparticles (SeNPs) are at the center of some intense biomedical researches due to their high biological activity and significantly lower toxicity compared to other forms of elemental selenium or its compounds. These particles are appreciated for their ability to annihilate free radicals *in vitro* and *in vivo*. *In vivo* effects are not only reduced to antiradical activity, but are manifested by numerous benefits for macroorganism, such as improving growth and reproduction performance. Currently, there are results demonstrating that biosynthesis of selenium nanoparticles can be achieved using different cultures of microorganisms or extracts from plants and algae. It is also shown that the coating of selenium nanoparticles with different organic molecules (amino acids, polysaccharides) leads to enhance their biological effects.

In this study, a synthesis system was used to produce selenium nanoparticles containing sodium selenite and polysaccharide fraction derived from biomass of cyanobacterium *Spirulina platensis*. In bionanosynthesis system involving selenium nanoparticles and polysaccharide extract from spirulina was established that changing the amplitude of absorption peaks started to rise after 30 min, which means the accumulation of a significant number of nanoparticles in the system. Over a time course of 120 min from the initiation of the reaction, a steady increase in the maximum absorption wavelength was observed, indicating the possibility of enhancing the yield of biosynthetic system at the expense of increased contact time.

It was determined the modification of reducing power of fractions depending on their contact time with sodium selenite. Reducing power of polysaccharide extract was 0.11 mg ascorbic acid per ml extract (equivalent), and after contact with selenium ions for 120 minutes it decreased by 89.1%. This parameter has decreased by about 22% compared with the period of 90 min contact.

Thus, biosynthesis of selenium nanoparticles on polysaccharide fractions derived from spirulina biomass was allowed to continue for 120 min followed by decreasing the reducing power of solution, which indicated the possibility of continuing the process.

INFLUENCE OF NANOPARTICLES OF CU, CO, ZNO ON MICROMYCETES

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Nanoparticles are used successfully in various fields due to their very small size and outstanding physicochemical properties. Inorganic nanoparticles are a focus of interest as stimulators and regulator of growth and biosynthetic processes of microorganisms producing bioactive compounds. The efficacy of their use can be both beneficial and toxic depending on the composition, size, duration and applied concentration.

The purpose of the researches was to study the influence of Cu, Co and ZnO nanoparticles on micromycetes. 20 strains of micromycetes from the National Collection of Non-pathogenic Microorganisms (CNMN) belonging to the genus: *Aspergillus* (5), *Trichoderma* (5) and *Penicillium*(10) were used as study material. Nanoparticles (NP) of Cu, Co and ZnO with the dimensions: NPCu - 2-3 nm, NPCo-15-20nm, NPZnO - 20-30 nm, were synthesized by the scientific researchers from Southwest State University,Kursk, Russia.

Micromycetes were grown on agar and liquid Czapek media with the following NP concentrations (%): Cu and ZnO - 0.001; Co - 0,0001. Cultivation on agar medium was carried out in Petri dishes at 28-30°C for 10 days. During this period the morpho-cultural peculiarities of the studied micromycetes were monitored (size, shape and color of the colonies, sporulation, spores, etc.). The submerged cultivation was carried out in 250 ml Erlenmeyer flasks on a shaker at 180-200 r.p.m. at 28-300° C for 6 days. As a control group, the variant without nanoparticles was investigated. The antifungal activity of the strains, belonging to the *Trichoderma* genus, was determined by agar blocks method, by the diameter of the growth inhibition zone of the phytopathogen. The following phytopathogens were studied as test-cultures: *Aspergillus niger, Alternaria alternata, Botrytis cinerea, Fusarium solani*, *Fusarium oxysporum*. The experiments were performed in 3 times. Statistical data processing was performed using MS Office Excel 2010.

The obtained results showed that Cu and ZnO nanoparticles added to liquid culture medium do not significantly change the micromycetes growth, the amount of biomass accumulated in the experimental variants varies within the limits ± 10% in comparison with control variant. The presence of Co NP in the growing medium significantly diminishes the growth of the micromycetes.

When grown on agar medium, NP of Cu and ZnO stimulated the growth and modified the mycelium color, and NP of Co inhibited growth in comparison with control group.

The study of the antifungal activity of genus *Trichoderma* cultivated on media supplemented with nanoparticles showed that the tested nanopatricles did not significantly influence the biosynthetic processes of the studied strains. Thus, the metabolites obtained in the variants with addition of nanoparticles showed less antifungal activity on the phytopathogen *A. niger* compared to the control variant. The sensitivity of *Alternaria alternata, B. cinerea* and *F. solani* was at the level of the control. A more pronounced sensitivity was shown by *Fusarium oxysporum* to the metabolites obtained in variants with Cu and ZnO nanoparticles, the inhibition zones exceeding the control by 4.5-12.8%.

According to the obtained results we can conclude that the supplementation of the cultivation medium with Cu and ZnO NP has not significantly influenced the growth, morphocultural properties and antifungal activity of the studied strains; and Co NP have an inhibitory effect on micromycetes growth.

PRODUCTIVITY AND CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS DURING *SPIRULINA PLATENSIS* CULTIVATION IN THE PRESENCE OF GOLD NANOPARTICLES (AUNPS)

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Gold nanoparticles (AuNPs) have wide applications in various nanotechnology fields, due to their unique properties and surface functionality. This multi-functionality of AuNPs provides a versatile platform for various nanobiological systems with respective oligonucleotides, antibodies, proteins. As a new concept, called "theranostics," AuNPs as multifunctionals may exhibit diagnostic and therapeutic functions that can be integrated into a single system, thereby facilitating the diagnosis, therapy and monitoring therapeutic responses.

It was studied biological effect of AuNPs on cyanobacterium *Spirulina platensis* growth and established the level of biomass accumulation. According to the obtained results, the effect of gold nanoparticles on spirulina growth was positive and this effect was provided by all doses added to culture medium. Thus, spirulina productivity in the presence of applied AuNPs doses increased by 18.75-34.3% compared to non-exposed biomass. Maximum biomass productivity increase by 32.29-34.3% compared to spirulina grown in the absence of nanoparticles, was recorded for AuNPs applied in dilutions of 1:1 (Au 1:1), 1:5 (Au 1:5) and 1:20 (Au 1:20).

Applying AuNPs did not lead to alteration of protein content in spirulina biomass. Although the protein content was lower by about 6-13% relative to the level of these biologically active compounds in spirulina biomass cultivated in the absence of AuNPs, we mention that the content of 64.65-69.75% was an appreciable one, framing in protein amounts characteristic of this culture.

The presence of AuNPs in spirulina cultivation medium did not essentially influence either the process of polysaccharide biosynthesis by this culture. The content of polysaccharides at all doses of gold nanoparticles registered values within the level of these functional compounds in biomass obtained by cultivating spirulina in the absence of AuNPs.

A more pronounced biological effect of AuNPs was set on the process of lipid biosynthesis by spirulina culture. Thus, lipid content increased in biomass. The highest level of lipidogenesis was determined for AuNPs in dilutions of 1:1, 1:5, 1:10 and 1:20, the increase in lipid amount constituting 24.33-28.22% relative to lipid level in non-exposed spirulina biomass.

Therefore, gold nanoparticles (AuNPs) are non-toxic for spirulina cultivation. These nanoparticles do not induce essential changes in protein and polysaccharide content of spirulina biomass. AuNPs stimulate strain growth by increasing the productivity by about 1.3 times and lipid content by about 1.25 times in biomass. Thus, AuNPs may be included in integrated schemes for the production of spirulina biomass - a source of nutraceutical compounds and these bionanoparticles.

THE EFFECTS OF ZNO NANOPARTICLES ON YEASTS CELL COLONIES: DIMENSION AND MORPHOLOGICAL PROPERTIES

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During recent years, zinc oxide nanoparticles (ZnO NPs) are utilized in different industrial sectors such as pharmaceutical and cosmetics industry, food and chemical industry. Zinc oxide NPs are a promising platform for use in biomedical research due to their anti-cancer, anti-bacterial properties. ZnO nanoparticles have gained interest in different applications based on their high stability, high specific surface area, nontoxicity, electrochemical activity.

Evaluation and determination of nanotoxicity are important for the safe application and environmental risk assessment of nanoparticles. In spite of, ZnO NPs have been reported to possess low toxicity for diverse microorganisms. In the present study, the effect of ZnO NPs was evaluated in pigmented yeasts *Rhodotorula gracilis* CNMN-Y-03.

In this paper are presented some experimental results of zinc oxide NPs-induced changes in yeasts cell colonies. It was established the effect of two types of nanoparticles ZnO <50 nm şi ZnO <100 nm on dimension and morphological properties of *Rhodotorula gracilis* CNMN-Y-03 strain depending on used concentration and contact duration. Commercially available ZnO nanopowder, (Sigma-Aldrich; particle size 50 and 100 nm) was used in the preparation of experimental solutions in concentrations from 1.0 to 70 mg/l. Research was effectuated on the solid fermentation medium YPD specific for yeasts [Aguilar-Uscanga et,al., 2003].

To obtain the colonies, yeasts cells of the control and experimental samples with NPs contact duration of 6 and 24 hours were seeded on solid YPD medium. The control culture is characterized by colonies 1-3 mm in size, R-shaped, with corrugated margins and intense coral color. Regarding the morphology of culture colonies in contact with nanoparticles ZnO <100 nm and ZnO <50 nm, some changes have been detected. There is also a significant difference in the dimension and color of colonies. It was observed that the color of the colonies of the experimental samples changes from intense to pale orange (specific for all concentrations ZnO nanoparticle concentrations <100 nm) and to pale orange and pink (specific for all concentrations of nanoparticle ZnO < 50 nm). That is supposed to be talked about the influence of nanoparticles on synthesis of pigments, in particular, of carotenoids.

Determination of the diameter of colonies demonstrated a difference between experimental and control samples. The presence of nanoparticles of ZnO <100 nm and ZnO <50 nm slightly stimulated the growth of yeasts colonies. It can be mentioned that the presence of nanoparticles ZnO <50 nm contributes to increasing of the dimension of the colonies in a higher degree, than of ZnO <100 nm.

In summary, yeasts colony morphology may be an indicator of nanoparticles action on microorganisms, this being an important adaptive process to overcome induced stress. The results demonstrated that ZnO NPs could influence the morphogenesis and depends on size of these particles. Furthermore, alterations in colony morphology may reflect increased resistance of yeasts.

EFFECTS OF IRON OXIDE NANOPARTICLES ON ANTIOXIDANT ENZYMES ACTIVITIES OF PIGMENTED YEASTS

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Over the past decades, nanoparticles (NPs) of metal oxides are widely used in different fields, such as medicine, food, pharmaceutical and cosmetic industries. Iron oxide nanoparticles have a large surface area, high reactivity, novel magnetic properties. Furthermore, when compared to other metallic nanoparticles, the iron oxide NPs are less expensive, harmless, biocompatible and less toxic.

However, there is a need to study the biochemical mode of iron oxide NPs action. As a redox metal, Fe participates in Fenton and Haber–Weiss reactions and the formation of reactive oxygen species (ROS) and oxidative stress. By modification of oxidation states, iron oxide nanoparticles further activate species like hydrogen peroxide (H_2O_2) and superoxide (O_2^-) to the highly reactive hydroxyl radical. The cellular defense system against toxicity induced by ROS includes superoxide dismutase (SOD), catalase (CAT) and glutathion peroxidases (GPx).

In this study, the effects of different concentrations from 0.5 to 30.0 mg/l of Fe_3O_4 NPs on antioxidant activities of pigmented yeast strain *Rhodotorula gracilis* CNMN-Y-30 were investigated. Commercially available Fe_3O_4 nanopowder, (Sigma-Aldrich; particle size 50-100 nm) was used in the preparation of experimental solutions. Research was effectuated on the solid fermentation medium YPD specific for yeasts.

Regarding the study of superoxide dismutase activity in yeast *Rhodotorula gracilis* CNMN-Y-30 under the influence of Fe_3O_4 (50-100 nm) NPs, the significant increase was recorded with the first used concentration of 0.5 mg/l. The maximum SOD activity compared to control (by 90%) was achieved by the introduction of iron nanoparticles at concentration of 10.0 mg/l. Subsequent increases of concentrations contributed to a slight decrease of SOD activity. At the same time, the enzyme values prevailed control values by 78-37%.

According to the results, Fe_3O_4 (50-100 nm) NPs influenced the activity of other antioxidant enzyme - catalase. The adaptive reaction response of *Rhodotorula gracilis* CNMN-Y-30 strain to the introduction of Fe_3O_4 nanoparticles (50-100 nm) was expressed by increasing the activity of catalase at concentrations of 0.5 ... 5.0 mg/L by 25 - 35%. The utilization of concentrations up to 30 mg/L contributed to the decrease with 5-50% compared to control. This can be explained by the excessive formation of reactive hydroxyl radical at high concentration of NPs. These results suggest that iron nanoparticles might exert an influence on the level of expression of SOD and CAT activity. Although, there are many antioxidant defense actions against oxidative stress, SODs constitute a significant mechanism among others.

This study reports that Fe_3O_4 NPs induced oxidative stress, as indicated by a significant increase of superoxide dismutase and catalase enzyme activities in pigmented yeasts. These studies suggest that iron oxide nanoparticles contributed to ROS formation, which alter the protein metabolism via reactive oxygen species formation.

THE EFFECTS OF IRON OXID NANOPARTICLES ON CAROTENOID PIGMENTS CONTENT OF YEAST *RHODOTORULA GRACILIS* CNMN-Y-30

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The development of science and technology during the past decade is characterized by intensive studies of the properties of nanoparticles and various ways for their practical application. Analyzing and generalizing studies, as well as a number of successful implementations of nanoindustry products, suggest that further progress in this direction will help to solve many problems facing humanity today. At the same time, there is no doubt that widespread application of nanotechnologies means the transition to a new level of scientific and technological progress in which its reverse side is full of new dangers associated with manifestations of properties and characteristics that were not yet known.

Metal nanoparticles are among the most widely used, especially Fe_3O_4, which are of interest for practical applications. Therefore, the adverse effects of Fe_3O_4 nanoparticles must obviously be investigated on cellular organisms in order to assess the risks. As attractive model microorganisms for studying the influence of nanoparticles on molecular and cellular mechanisms, strains of the genus *Rhodotorula* can be used effectively for this purpose. Due to the cellular structure, functional organization and ability to synthesize antioxidant compounds such as carotenoid pigments, this unicellular eukaryotic organism can serve as a model for assessing cellular responses to nanoparticle toxicity and subsequent elaboration of detoxification strategies.

In this context, the purpose of the research was to evaluate the effects of iron oxide nanoparticles on carotenoid content of *Rhodotorula gracilis* CNMN-Y-30 yeast strain.

For impact assessment in the experiments we studied the changes induced by Fe_3O_4 nanoparticles with size of 50-100 nm (Aldrich product) with different concentrations of 0.5; 1.0; 5.0; 10.0; 15.0; 20.0; 25.0; 30.0 mg/L, on biosynthetic potential for carotenoid accumulation and β-carotene, toruline, torulorodine base components in *Rhodotorula gracilis* CNMN-Y-30 strain.

According to the obtained results, the yeast response to the presence of nanoparticles was manifested by an increased toxicity effect on carotenoid pigment biosynthesis.

Under the influence of concentration ranges 0.5...30.0 mg/L, it was observed a decrease of carotenoid content by up to 72.9% compared to control sample.

To determine the degree of influence of nanoparticles on carotenoid content, the concentration was determined spectrophotometrically. The data indicated that the content of these pigments decreased directly proportional to the concentration of Fe_3O_4 nanoparticles (50-100 nm) introduced into YPD cultivation medium. Therefore, we can assume that Fe_3O_4 nanoparticles lead to blocking the carotenoid biosynthetic processes, which have been manifested by major changes in their content in yeast biomass. Using high concentrations of nanoparticles, the yeast cells may completely lose their pigment biosynthesis ability.

By generalizing the results we can state that Fe_3O_4 nanoparticles (50-100 nm) have a high toxicity effect that places them in the hazard category - harmful substances.

BIOSYNTHESIS OF POLYSACCHARIDES IN *RHODOSPORIDIUM TORULOIDES,* CULTIVATED IN THE PRESENCE OF FE_3O_4 (50-100 NM) NANOPARTICLES

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Microbial polysaccharides are located in the cell membrane; the polymerization degree and chemical structure are individual characteristics of each strain. The functionality of yeasts polysaccharides, mostly composed of β-glucans, mannoproteins, chitin depends on the cultivation conditions. From a biotechnological point of view, the efficiency of polysaccharides production can be increased by stimulating the biosynthesis process or by achieving greater productivity of cellular biomass.

In this paper are presented the results of Fe_3O_4 (50-100 nm) nanoparticles in concentrations from 0.5 mg/L to 30 mg/L on the cell biomass and polysaccharides production in *Rhodosporidium toruloides* CNMN-Y-30. Fe_3O_4 nanoparticles (SIGMA-ALDRICH product) in the form of powder, surface area> 60 m^2/g, density 4.8-5.1 g/ml at 25^0C refer to the category of biomedical materials

Analysis of data on the influence of Fe_3O_4 (50-100 nm) nanoparticles on cell biomass production revealed that after 120 hours of contact, the yeast reacted positively to the concentrations applied in the experiments. According to the studies, nanoparticles at concentrations of 0.5 - 30 mg/L exhibit stimulatory effect on cell biomass production, the highest 24% increase compared to the control was recorded for the 20 mg/L concentration.

The nanoparticles influence on polysaccharide biosynthesis processes is evident, depending on the concentrations applied in the nutritive medium. The obtained results demonstrate that the polysaccharides amount in yeast biomass at cultivation of strain in the presence of 0.5-5 mg/L nanoparticles practically has not changed compared to the control samples. Administration of higher concentrations of nanoparticles substantially reduced the polysaccharides production. Research has revealed that nanoparticles used in the culture medium at concentrations of 25 and 30 mg/L reduced the polysaccharide content in yeast biomass by 35.2...37.2% compared to the control.

To determine the role of nanoparticles concentrations in the process of polysaccharides accumulation, correlation analysis was performed. The correlation between the amount of polysaccharides and nanoparticles concentrations used for the cultivation of *Rhodosporidium toruloides* CNMN-Y-30 determined a strong dependence between the values of these parameters, (R^2 = 0.8359), therefore in 83 cases the concentration of nanoparticles determines the accumulation of polysaccharides in the microbial cell.

In conclusion of the study, Fe_3O_4 nanoparticles with 50-100 nm sizes in concentrations from 0.5 to 30 mg/L affected biochemical systems of polysaccharide synthesis and their effect depends on the used concentration. In perspective, these results may contribute to the development of theoretical and practical bases in the context of studies on the use of Fe_3O_4 nanoparticles in nanobiotechnology.

ESTIMATION OF CELL VIABILITY AT THE CONTACT OF YEASTS WITH ZNO AND FE_3O_4 NANOPARTICLES

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Cell viability tests carried out by the determination of colony-forming unit (CFU) are increasingly used to determine the toxicity of different types of chemical compounds, nanocomposites or biomaterials. In microbial biotechnology, for functional tests of the degree of inhibition or stimulation of a compound observed in dose - response reaction, it is proposed to use the terms "half maximal effective concentration" (EC_{50}) or "half maximal inhibitory concentration" (IC_{50}) of the compound. These parameters provide an opportunity to conduct a comprehensive study of nanoparticle toxicity.

The present research has been focused on estimating the effects of ZnO nanoparticles with dimension <100 nm and Fe_3O_4 with dimension of 10 and 30 nm on cell viability of two yeast strains of the genus *Saccharomyces* and *Rhodosporidium* .

Analyzing the results obtained at 24 hours of contact of *Saccharomyces cerevisiae* CNMN-Y-20 strain with ZnO (<100 nm) nanoparticles, it was found that the viability rate practically did not change. For concentrations of 0.5-15 mg/L, the viability rate was between 96-87.5% compared to control. *Rhodosporidium toruloides* CNMN-Y-30 strain samples demonstrated modifications in cell viability under the influence of ZnO (<100 nm) nanoparticles in concentrations of 1-30 mg/L. Relevant results were observed at yeast contact with ZnO nanoparticles in concentrations of 20-30 mg/L, when the percentage of viable cells decreased to 59%. The obtained results can be summarized as follows: ZnO (<100 nm) nanoparticles in concentrations from 0.5 mg/L to 30 mg/L added to YPD culture medium induced some variations of the viability of yeasts *Saccharomyces cerevisiae* CNMN-Y-20 and *Rhodosporidium toruloides* CNMN-Y-30. For nanoparticles concentrations used in our experiments, the half maximal effective concentration (EC_{50}) and half maximal inhibitory concentration (IC_{50}) have not been recorded.

Exploratory data analysis of the action of Fe_3O_4 nanoparticles with dimensions of 10 and 30 nm showed that the rate of viability varied depending on their size and concentration. The determination of colony-forming units (CFU) for *Rhodosporidium toruloides* CNMN-Y-30 strain at 24 hours of contact with 10 nm Fe_3O_4 nanoparticles in concentrations of 0.5-30 mg/L has revealed a high sensitivity of yeast to the action of iron oxide nanoparticles. The toxic effects were evident at concentrations of 20, 25 and 30 mg/L, when viability decreased to 40-32% compared to control. Low viability values were noted in the experiments where yeast cells were in contact with 30 nm Fe_3O_4 nanoparticles. Concentrations of 25-30 mg/L reduced cell viability to 75-60% compared to control. Thus, half maximal inhibitory concentration (IC_{50}) was specific for Fe_3O_4 nanoparticles of 10 nm at 20 mg/L concentration.

Thus, the test for yeast viability by determining colony forming units (UFC) is highly reliable, but, in some cases, the interpretation of the results may be inaccurate due to errors in serial dilutions or inoculation of biological material on Petri dishes. In this context, other procedures of nanoparticles toxicity evaluation, such as determination of oxygen consumption, impact on cell membrane integrity, antioxidant activity are also recommended.

IMPACT OF NANOPARTICLES ON THE MORPHOLOGY OF MYCELIAL FUNGI AND ITS RELATION TO ENZYME BIOSYNTHESIS

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In submerged culture processes, filamentous microorganisms typically have a complex morphology during their life cycle that is related to production performance – a relation that is of high interest for optimization process. The morphology is strongly influenced by process parameters, including mechanical stress due to stirring and aeration, pH level, composition of the culture medium (Nielsen et al., 1995; Papagianni, 2004; Bizukojc and Ledakowicz, 2010, Wucherpfennig, 2012), osmolality and the presence of solid microparticles (Krull et all, 2013; Kaup et all, 2008; Driouch et all, 2011).

The aim of this review was to present the impact of nanoparticles on morphological engineering techniques in the cultivation of filamentous fungi.

Under submerged cultivation on agarized media, it was revealed the ability of MgO, ZnO, MgO/ZnO, TiO_2 and Fe_3O_4 nano-oxides with different physicochemical characteristics to induce morphological changes in mycelial fungi from the genera *Fusarium, Trichoderma, Rhizopus* – producers of exocellular hydrolases.

The addition of 30 nm ZnO nanoparticles and 65-70 nm Fe_3O_4 nanoparticles, selected in prior researches as effective stimulators of exocellular protease biosynthesis in *Fusarium gibbosum* CNMN FD 12 and *Trichoderma koningii* CNMN FD 15 strains, resulted in appearing the colonies with distinct morphology, differing in size, color and mycelium structure on the agar medium.

In order to enhance the biosynthetic activity of producer strains, it was revealed the opportunity to use as seed material cultures derived from morphologically modified colonies under the influence of nanoparticles. The utilization of morphologically modified colonies under the influence of ZnO and Fe_3O_4 nano-oxides as inoculum at submerged cultivation increased the activity of neutral proteases by 192.6% and 48.1% in *Trichoderma koningii* CNMN FD 15 and *Fusarium gibbosum* CNMN FD 12 strains, respectively.

Fe_3O_4 nanoparticles measuring 70 nm did not change the morphology of *Rhizopus arrhizus* CNMN FD 03 culture. However, Fe_3O_4 nano-oxide exerted an essential positive influence on fungal metabolism, resulting in increasing the lipolytic activity by 248.8% (3.5 times) and appearing the biosynthesis absorption peak on the second day of cultivation, being with 24 hours earlier than the previous assessment model.

Iron nano-oxide ensured the expansion of stationary growth phase of micromycete *Rhizopus arrhizus* CNMN FD 03. Moreover, stimulatory effect was significantly higher than control (with 45.8-248.8%) throughout the overall cultivation cycle (days 1-4).

IMPACT OF FE^{++} NANOPARTICLES ON THE DEVELOPMENT OF LUCERNE TREATED WITH TRIFLURALIN HERBICIDE

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Moldova has become a predominantly agrarian country, due to the favorable natural conditions, that allow obtaining high quality products, which makes it compatible in this respect on the world market. However, the expected crop yield depends on several factors, including the use of herbicides as a necessary remedy, on the other hand – damaging the environment.

Under the above-mentioned aspect, research to reduce the negative effect of these substances has a priority character. Particular attention has been paid to the use of nanotechnology based methods in several countries in recent years. Investigations have shown that nanometric iron oxide particles are extremely effective in binding and removing arsenic from groundwater that affects the water supply of millions of people and for which there is no effective solution.

Based on the above, our team of Soil Microbiology Laboratory starts from 2015 the researches to identify ways to intensify the bioremediation of soil polluted by pesticides using nanoparticles.

Research purpose of this study was to identify the ability of iron nanoparticles to stimulate the interaction between nitrogen-fixing symbiotic microorganisms (*Rhizobium meliloti* 2/13) and the lucerne in soil conditions polluted with the herbicide trifluralin.

The experiments were carried out for 12-17 days under climatic conditions with artificial lighting at 22-25^0 C. To perform the experiments we used Petri dishes with unpolluted soil, where the pre-processed lucerne seeds were introduced with the *Rhizobium meliloti* 2/13 suspension. Additionally, the soil was wetted with aqueous solution of Trifluralin (each time according to the planned concentration – from 1 to 400 mg/l). Fe^{++} nanoparticles were also used in experiments.

As a result of the research, the following conclusions were made:

1. Trifluralin used in doses: 1, 10 and 20 mg/l adversely affect the development of lucerne plants. These doses do not have a negative effect on seed germination.

2. Trifluralin doses of 50, 100 and 200 mg/l insignificantly affeced the growth of bacteria *Rhizobium meliloti* 2/13.

3. Introduction of Fe^{++} nanoparticles (25 mg/l) favored the neutralization of high-dose inhibition of trifluralin (400 mg/l) in *Rhizobium meliloti* 2/13 bacteria.

4. Obtained data demonstrated that the herbicide trifluralin exerted a negative influence on the growth and development of plants and of the symbiotic-nitrogen-fixing microorganisms (*Rhizobium meliloti*).

5. Data obtained also shows that Fe^{++} nanoparticles have a positive influence on the growth and development of lucerne plants and the symbiotic-nitrogen-fixing microorganisms (*Rhizobium meliloti* 2/13).

EFFECT OF COPPER NANOPARTICLES (CUNPS) ON GROWTH AND BIOCHEMICAL COMPOSITION OF CYANOBACTERIUM *SPIRULINA PLATENSIS*

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Copper is 3d orbital transition metal with distinct physical and chemical properties. Cu-based materials can promote and produce a variety of reactions due to accessible oxidation states (Cu, Cu I, Cu II and Cu III). Due to the distinct properties of this metal, copper nanoparticles (CuNPs) have found numerous applications in nanotechnology. Cu-based nanocatalysts are currently applied in catalytic organic transformations, electrocatalysis and photocatalysis. Biological properties of copper determined applying CuNPs as antibacterial, imaging and immunomodulating agents.

It was studied the effect of 5 nm size copper nanoparticles (CuNPs) on growth and biochemical composition of cyanobacterium *Spirulina platensis*. The presence in cultivation medium of CuNPs in concentration of 0.5 mM led to a decrease of spirulina productivity by about 13%. Spirulina productivity increased on its cultivation in the presence of lower doses of CuNPs (from lower to highest dilutions in cultivation medium). The highest biomass production level was reached at the lowest dose of CuNPs applied to spirulina culture (1:20 dilution). This dose enhanced spirulina productivity by approximately 26% compared to spirulina cultivation in the absence of CuNPs.

CuNPs showed a pronounced toxic effect on protein biosynthesis in spirulina that was specific in particular to the first doses of nanoparticles. In this case, protein content decreased with 17-29%. Only in the presence of lower doses of CuNPs (1:10 and 1:20 dilutions), protein content recorded values within the limits of protein content characteristic of non-CuNPs biomass.

The content of polysaccharides changed insignificantly on spirulina exposure to CuNPs. A decreasing trend in polysaccharide content was characteristic for all applied CuNPs doses. Thus, the level of polysaccharides in spirulina biomass decreased by about 5-13%. On the background of decreasing protein and polysaccharide content, lipid quantity was lower in the presence of the first two doses of CuNPs, but was maximally altered by 1:5 dilution and slightly decreased at higher dilutions of CuNPs. The highest polysaccharide content was obtained in biomass exposed to CuNPs in 1:5 dilution and constituted 7.43%, which was about 52% more compared to lipid level in algal biomass not subjected to the effect of copper nanoparticles.

Therefore, CuNPs exhibited an effect from stimulative (biomass productivity and lipids) to toxic (proteins) on the growth and levels of biologically active compounds from *Spirulina platensis*. Taking into account that the levels of biologically active compounds from spirulina grown in the presence of CuNPs were not significantly diminished compared to those characteristic of this culture, biomass produced in the presence of CuNPs could be used as raw material for nutraceuticals containing bionanoparticles with antibacterial effects.

EFFECT OF SILVER NANOPARTICLES (AGNPS) ON *SPIRULINA PLATENSIS* PRODUCTIVITY AND CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS

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At present, silver nanoparticles (AgNPs) are used in numerous technologies and are being incorporated into a wide range of consumer products, which are based on optical, conductive and antibacterial properties of these nanoparticles. An obvious area is AgNPs applications in molecular diagnostics as biosensors and numerous tests, where nanostructured materials can be used as biological markers for quantitative detecting. An important aspect of applying AgNPs is based on their activity as biosensors and regulators of plant metabolism. Due to the fact that it does not require organic media, technologies involving photosynthetic organisms are far less expensive. Biological effect of silver nanoparticles in particular upon microalgal and cyanobacterial metabolism is poorly studied. For these reasons, the study of the effect of AgNPs on biosynthesis of biologically active compounds by cyanobacteria and microalgae and the ways of enhancing the yield of this process is a perspective field of nanobiotechnology.

In our study, 5 nm size AgNPs were used to cultivate cyanobacterium *Spirulina platensis*. It was determined the beneficial effect of these nanoparticles on spirulina growth through enhanced productivity by about 22-30%. Maximum biomass content of 1.13 g/L was obtained in the presence of these nanoparticles in 1:5 dilution, which was 30% more.

The process of protein biosynthesis was virtually unaffected by the presence of AgNPs. Thus, the protein content oscillated in biomass within the limits of protein content characteristic of this strain.

A similar picture was also characteristic for polysaccharides. These functional compounds were relatively inert to AgNPs presence. As in the case of proteins, polysaccharide content remained in the area of characteristic values for this spirulina strain.

Another situation was lipid dynamics in spirulina grown in the presence of AgNPs. Lipid content was lower by about 16% at concentration of 0.5 mM of AgNPs, and had an increasing trend at dilutions of 1:5, 1:10 and 1:20 of this AgNPs concentration. An increase in lipid content with about 52% was produced in spirulina biomass at 1:1 dilution of AgNPs.

Therefore, AgNPs exhibit a varied biological effect on the productivity and content of biologically active compounds from *Spirulina platensis*. Namely, an effect from stimulative (productivity and lipids) to adaptogenic one: it does not change protein and polysaccharide content. Since the levels of compounds from spirulina grown in the presence of AgNPs are comparable to those characteristic of this culture, obtained biomass could be used as raw material for nutraceuticals.

GENOME PHYLOGENY OF GENUS RHIZOPUS

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Phylogenomic approach has the tremendous potential to resolve the inter-relationships of the fungal taxons in the order *Mucorales* within the tree of life. Species of genus *Rhizopus* are especially important as animal and plant pathogens and industrial fermenters for food and metabolite production. They are ubiquitous soil dwellers and their spores can be found literally in any soil, plant or aerial environmental sample. A dataset of 192 orthologous genes (single copy genes) was used to construct a phylogenetic tree of 21 *Rhizopus* strains, classified into four species isolated from habitats of industrial, medical and environmental importance. The phylogeny indicates that the genus *Rhizopus* consists of three major clades, with *R. microsporus* as the basal species and the sister lineage to *R. stolonifer* and two closely related species *R. arrhizus* and *R. delemar*. A comparative analysis of the mating type locus across *Rhizopus* reveals that its structure is flexible even between different species in the same genus but shows similarities between *Rhizopus* and other mucoralean fungi. The topology of single-gene phylogenies built for two genes involved in mating is similar to the phylogenomic tree. Comparison of the total length of the genome assemblies showed that genome size varies by as much as threefold within a species and is driven by changes in transposable element copy numbers, genome duplications and possibly hybridization.

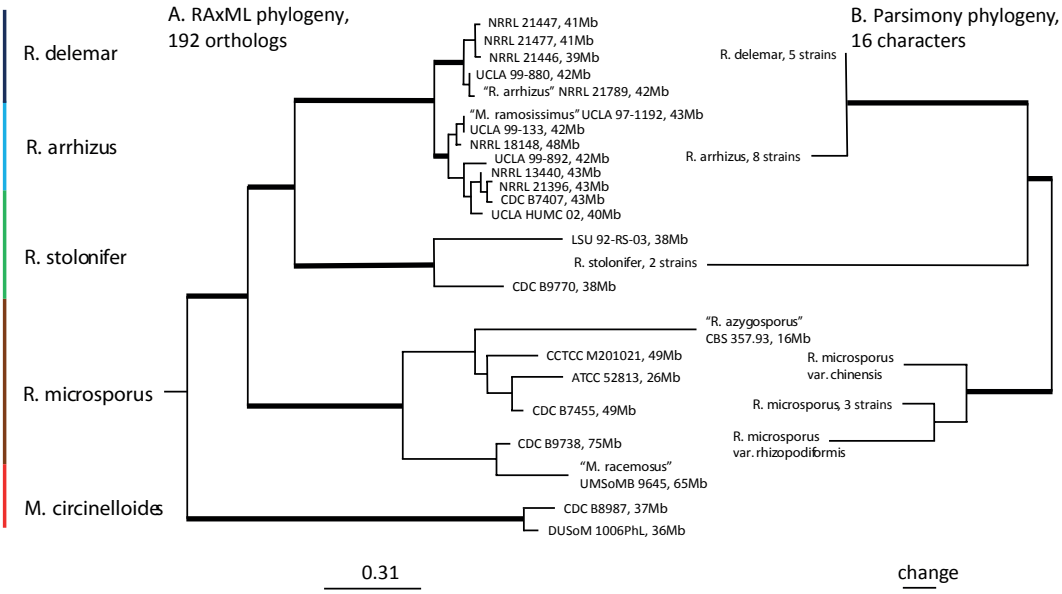

Fig. Genome-based maximum likelihood phylogeny and parsimony phylogeny based on non-molecular characters. (A) Rooted maximum likelihood tree of the genus Rhizopus based on 192 orthologous genes. Misidentified strains are indicated in quotes: "Mucor racemosus" B9645 = R. microsporus B9645 and "Mucor ramosissimus" 97-1192 = R. arrhizus 97-1192. Genome size is indicated in bold after the strain name. (B) Unrooted parsimony tree of 16 non-molecular (14 micromorphological and two ecological) characters. Morphological and physiological data for different strains of the same species are consolidated in the tree except for those strains that differ in at least one character. Thick branches denote statistically significant bootstrap values.

SELECTION OF *PICHIA PASTORIS* STRAINS – PRODUCERS OF HORSERADISH PEROXIDASE ISOENZYME C2

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Peroxidases (EC 1.11.1.X) are the most popular enzymes in analytical chemistry and medical diagnostics. Usually plant peroxidases are used in common practice. The best studied enzyme is the horseradish peroxidase (HRP) C1A. The recovery of peroxidases from plant sources entails some difficulties in enzyme purification because of enzyme content dependency on cultural conditions. These disadvantages led to the necessity to find alternative peroxidase sources.

In recent years the progress made by genetic engineering in cloning of eukaryotic genes in heterologous systems open up new opportunities for selection of producers of this enzyme.

Now there are several HRP expression systems. Expression in *E. coli* often serves to obtain recombinant HRP. In this case the enzyme is derived in an insoluble and inactive form. Refolding and reactivation is time-consuming and multi-step process. The secretion of HRP in the periplasm of *E. coli* cells results in small amounts of active and soluble enzyme. Recently, yeast systems have been engaged to obtain active soluble HRP.

The aim of this work was to construct a vector for the expression of the gene encoding HRP C2 in *Pichia pastoris* cells and to obtain transformants synthesizing this enzyme.

The plasmid pPICZαA was used for constructing the expression vector in *P. pastoris*. The gene HRP C2 was synthesized artificially by Eurofins Scientific Company. The codon optimization for gene expression in *P. pastoris* was carried out previously. Plasmid pPICZαA-HRP was engineered. The restriction analysis of the plasmid, shown in the figure, was performed using EcoRI restriction enzymes (the size of the DNA fragments after restriction was 3593 bp and 1004 bp), HindIII (plasmid size 4597 bp), and BamHI (3702 bp, 475 bp, 420 bp) and the necessary genetic construction was selected.

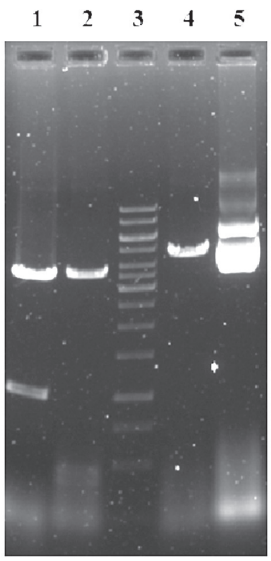

Fig. – Electrophoregram of plasmid DNA pPICZαA-hrpC2 (1-plasmid pPICZαA-hrpC2, EcoRI restriction site, 2-plasmid pPICZαA-hrpC2, BamHI restriction site, 3 - molecular weight marker (GeneRuler ™ 1˚kb˚DNA Ladder, ThermoScientific, Lithuania)); 4 - plasmid pPICZαA-hrpC2, HindIII restriction site; 5 - plasmid vector pPICZαA-hrpC2

Thus, the recombinant plasmid DNA pPICZαA-hrpC2 was obtained, and further inserted into the yeast strains - producers of HRP C2. The resulting transformants synthesized hrp in active form.

Grey Biotechnology

Classical fermentation and Bioprocess Technology

INFLUENCE OF VEGETAL IRIDOID GLYCOSIDES ON THE VIABILITY OF STREPTOMYCES AFTER LYOPHILIZATION

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Currently, due to increasing demand, researchers from different areas of biology emphasize the need to obtain new natural substances with high biological activity. In in recent years, vegetal iridoid glycosides extracts have been studied more and more frequently, in this context.

Thanks to immunomodulatory, antiinflammatory, antihepatotoxic, choleretic, hypoglycemic, antispasmolytic, antioxidant activity, these substances are used for the preparation of various pharmaceuticals. Iridoid glycosides are increasingly used in plant protection, due to their antiviral, and adaptogenic properties; positive influence on seed germination, tissue regeneration and cell membrane protection by reducing lipid peroxidation. Some of these properties justify the use of vegetable glycosides as lyoprotectants for the preservation of microorganisms. Choosing the right methods for micro-organisms preservation is one of the main tasks of microbial culture collections. Currently, freeze-drying and cryopreservation are considered by researchers the most effective and long-lasting methods of preserving strains of scientific and biotechnological interest.

The genetic stock of industrial microorganisms represents for Moldova an inexhaustible natural resource, the national patrimony. Storing, maintaining viability and long-lasting stabilization of taxonomic characters of microorganisms with beneficial scientific and technological use, is the main objective of the CNMN and of all collections all around the world.

According to the literature data, the microorganism viability reduction during preservation could be induced by the free radicals formation. Some authors consider that their appearance is determined by both the change in the ratio between different forms of water in the cell and the destruction of the polypeptide chains. According to other opinions, this process takes place due to oxidation reactions. Thus, both free radicals and oxidative lipid products, interacting with cell membranes, lead to lesions causing the cell death. In order to avoid cell death during lyophilization, the used protective media usually contain substances with cryoprotective properties, membrane stabilization and antioxidant activity.

Based on the above, the purpose of the research was to evaluate the influence of vegetal glycosidic extracts from *Verbascum phlomoides* (L.) and *Linaria genistifolia* (L.) Mill. on the viability of streptomycetes after lyophilization and long-term storage.

The subject of the study was the strain *Streptomyces canosus* CNMN-Ac-02. The above mentioned glycosidic extracts in concentrations of 0.00005-0.05% / V were tested as lyoprotectors added to the protective medium - 2.5% gelatin + 7.5% glucose (control medium).

In previous researches was demonstrated the lyoprotective effect of oligo-peptide and polysaccharide cyanobacterial extracts, which increase the viability of streptomyces up to 20.1-38.0% after lyophilization and up to 28.3-39.8% after 1 preservation cycle compared to the blank test. The efficacy of the iridoid glycosides proved to be even higher. Thus, supplementation of the standard medium with glycosides in concentrations up to 3 orders less than cyanobacterial extracts resulted in the increase of the studied strain viability relative to the reference samples up to 10.1% after lyophilization and up to 5.4% after 1 year of conservative use of verbascosides

and 18.0 and 16.6% respectively when using genistifoliasides. The innovative character of these researches is evidenced by the patents for invention (MD 1226, MD 1235).

Thus, we can conclude that glycosidic extracts positively influence the viability of streptomyces and can be used in the composition of protective media for the preservation of microorganisms by lyophilization. The results of these researches will serve to optimize the methods for the long-term preservation and storage of the CNMN microbial gene pool.

INFLUENCE OF SPIRULINA BIOACTIVE EXTRACTS ON THE BACTERIA LYOPHILIZATION

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The use of protective media in the process of lyophilization of microorganisms has an important function in the preservation for a long time of their characteristic properties during conservation. The polycomponent extracts obtained from spirulina biomass, used as natural preservatives in the lyophilization process of microorganisms, have shown their efficacy depending on the studied strain.

In this research, some biotechnologically valuable strains of bacteria, deposited in the National Collection of Nonpathogenic Microorganisms of IMB were selected. The obtained results on the viability of the pseudomonas strains established a stimulatory effect of all tested bioactive extracts, excepting the ethanol extracts with an insignificant decrease of viability (7.6 – 9.3%) after lyophilization (Table 1).

Table 1. Viability of bacteria strains before and after lyophilization in the presence of bioactive extracts from spirulina biomass

		Pseudomonas fluorescens CNMN-PsB-01		*Pseudomonas aurantiaca CNMN-PsB-08*		*Bacillus subtilis CNMN-BB-01*		*Bacillus cereus var. fluorescens CNMN-BB-07*	
	Conc.	**before**	**after**	**before**	**after**	**before**	**after**	**before**	**after**
Control		100,0±0,5	99,9±0,9	100,0±0,4	98,6±0,9	100,0±0,6	98,8±1,1	100,0±0,7	94,5±1,8
Ethanol extract 50%	5%	106,1±1,0	90,7±1,0	100,2±2,7	93,3±1,4	99,6±0,6	103,0±1,7	95,7±2,5	98,8±0,8
	10%	106,7±2,3	94,7±1,5	103,2±1,3	92,5±2,6	101,0±0,5	102,8±0,6	98,8±2,0	100,3±2,8
Ethanol extract 65%	5%	105,2±0,7	101,9±0,4	103,4±0,6	92,4±0,9	99,6±0,7	100,9±1,1	95,1±1,1	94,1±0,6
	10%	103,4±1,2	100,3±0,5	104,5±1,0	92,8±1,9	101,2±0,7	99,2±0,6	100,3±0,9	94,1±1,4
Protein extract	5%	101,4±1,3	102,0±0,8	105,2±1,3	102,6±0,2	101,7±0,8	99,5±0,7	98,7±2,2	102,2±1,5
	10%	100,6±1,9	104,7±0,9	103,6±0,8	98,9±0,3	102,4±1,3	103,6±0,5	100,3±0,9	99,7±1,8
Proteoglycan extract	5%	105,2±0,8	102,4±1,1	106,9±1,1	104,4±1,9	103,4±0,4	102,8±0,7	105,5±1,9	95,9±1,2
	10%	106,0±0,4	100,0±0,8	106,5±0,6	101,7±1,5	103,7±0,5	103,1±0,6	100,9±1,6	93,8±0,9
Carbohydrate extract	5%	109,2±0,9	108,6±1,0	107,1±0,9	106,6±1,3	102,4±0,6	100,2±0,4	103,6±0,9	98,0±2,1
	10%	106,5±1,0	106,2±0,6	104,1±0,3	102,9±1,5	101,6±1,0	98,4±0,9	103,4±0,9	98,4±1,6

The highest efficiency in the protection of pseudomonas cultures has been shown by the carbohydrate extract from spirulina biomass.

Another group of tested bacteria of biotechnological interest was representatives of genus *Bacillus.* The data obtained on the cultivation of the *Bacillus subtilis* CNMN-BB-01 in the presence of bioactive extracts before lyophilization, allowed concluding that all extracts had a positive effect.

The strain *Bacillus cereus var. fluorescence* CNMN-BB-07 responded differently to the presence of bioactive extracts in the medium. Thus, its viability before lyophilization with the use of ethanol extracts tended to a nonessential decrease with 4.9% compared to the control sample. Protein-carbohydrate (5%) and carbohydrate extracts (5 and 10%) had a stimulating effect on the viability of this strain. The results obtained after lyophilization demonstrated that the 5% protein extract had the best effect on the viability of *Bacillus cereus var. fluorescence* CNMN-BB-07, with 7.7% increasing compared to control.

BIOLOGICAL ACTIVITY OF *S. LEVORIS* CNMN-AC-01 AFTER LONG-TERM STORAGE BY SUBCULTURING AND UNDER MINERAL OIL

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Preservation of vitality and biotechnological properties of different types of actinobacteria is associated with certain difficulties due to the high level of their genetic instability. Different storage methods have their advantages and disadvantages.

The aim of the studies was to determine the effect of long-term storage (10 years) of strain *S. levoris* CNMN-Ac-01 by subculturing and under mineral oil.

It was found that during storage process by both subculturing and under mineral oil, the cultural properties of the strain practically did not undergo significant changes: in the colour of the aerial mycelium – a typical pinkish-paler shade predominated, and for substrate mycelium – yellowish one predominated during growth on classical Czapek media with glucose, Gause, or oat agar.

The amount of biomass after storage of the strain changed as follows: initially, when cultivated on a liquid complex medium M-I, the strain accumulated up to 6.29 ± 0.34 g/l. After long-term storage by subculturing, the quantity of biomass decreased to 5.08 ± 0.12 g/l, then in the variant under mineral oil - to 5.4 ± 0.2 g/l. The quantity of total lipids in biomass was initially 12.54%, after storage – 11.8% and 12.1%, respectively. Comparing the fractional composition of the total lipids in biomass after long-term storage by both methods, it was noted that qualitative and quantitative composition of the main lipid fractions remained unchanged: the number of basic physiologically important fractions as phospholipids was 13-14.47% and sterols 16.0-17.7% of the total amount of lipids, regardless of the storage method.

The ability to depress the growth of phytopathogenic test microorganisms (bacteria and fungi) has changed as follows: the activity against *Erwinia carotovora* was by 10.6-11.3% lower for the strain stored by subculturing, than for the same strain stored under mineral oil. The activity against *C. michiganensis* remained unchanged: up to 19.0-20.0 mm diameter of the growth inhibition zones. Also, no changes were noted regarding the phytopathogenic fungi *A. niger* and *A. alternata* (the size of the inhibition zones - 10.0-13.0 mm). A slight decrease was noted in activity against *B. cinerea* (15.0 mm – zone of growth inhibition at the beginning of studies and 13.0-13.5 mm after the storage by both methods).

Thus, the conducted studies showed that during long-term storage (10 years) of *S. levoris* CNMN-Ac-01 by subculturing or under mineral oil, its biosynthetic activity (accumulation of biomass during cultivation on nutrient media and its lipid content) and antimicrobial activity against some phytopathogens decreased.

THE AMINO ACID COMPOSITION OF *STREPTOMYCES FRADIAE* CNMN-AC-11 BIOMASS, CULTIVATED ON A COMPLEX MEDIUM WITH THE BIO PRODUCT BIOR

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Currently the microbiological industry serves as an important supplier of various amino acids (glutamic acid, lysine, methionine, etc.). Among microorganisms-producers one of the first places is occupied by actinomycetes, in particular, the genus *Streptomyces*. As a result of microbial relatively environmentally safe synthesis, L-forms of amino acids are produced. The enzymes for amino acid synthesis process can be also obtained by directed microbial synthesis.

The subject of the study was the strain *Streptomyces fradiae* CNMN-Ac-11, isolated from the soil of the central part of Moldova. The culture was stored in two ways: in a lyophilized form and by periodic transfer, using Czapek, Gause and oatmeal agar media. The investigated strain was grown in a liquid medium R with the addition of 0,1% BioR (an extract of amino acids and peptides of *Arthrospira platensis*). The amino acid composition of the obtained biomass was determined by ion exchange chromatography using the amino acid analyzer AAA-339 M "Microtehna" (Czech Republic).

Cultivation of the strain on the changed medium showed an increase in the amount of biomass, total protein and individual groups of amino acids. After addition of BioR at concentration of 0,1% the amount of biomass increased by 18%. In addition, a positive effect of BioR, added to the main medium, on the accumulation of the protein in the biomass was observed. The increase in protein yield was 23,5% compared to the control value. Analysis of the total amino acid content in the *Streptomyces fradiae* CNMN-Ac-11 biomass showed an increase in the yield of almost all amino acid groups, except for nonessential ones. The increase in the amount of essential amino acids was 78,2%, immunoactive – 14,2%, the amount of glycogenic amino acids increased by 40,6%, ketogenic – by 71,25%, proteinogenic – by 24,3% and sulfur-containing – by 55,5 %. Comparative analysis of the amino acid content in the biomass of *Streptomyces fradiae* CNMN-Ac-11 cultured on complex medium R with the addition of 0,1% BioR showed a marked increase in the amount of individual amino acids. For example, the amount of threonine increased by 109,26%, serine – by 112,1%, valine – by 102,1%, phenylalanine – by 114,5%, compared to the control sample of biomass. The increase in the amount of amino acids such as cysteine, leucine, isoleucine, lysine, histidine is less pronounced: the yield of cysteine increased by 78,86%, leucine – by 59,6%, isoleucine – by 61,5%, lysine – by 89,5%, histidine – almost 94%.

Thus, the obtained data allow considering the bio product BioR for increasing the biosynthetic activity of *Streptomyces* strains - producers of amino acids.

THE EFFICIENCY OF LONG-TERM STORAGE METHODS FOR *PENICILLIUM PICEUM* – MICROBIAL PRODUCER OF CATALASE, INTENDED FOR APPLICATION IN MEDICINE AND INDUSTRY

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Development of technologies for production of enzyme preparations by microbial synthesis necessitates to focus special attention on methods for preservation of industrial strains – producers. Stability of initial characteristics of microbial cultures ensuring possibility of their planned application must be maintained in the course of storage. When storing the producers of enzymes, a vital prerequisite is to preserve stable level of their biosynthetic activity.

One of the practically important enzymes is catalase (EC 1.11.1.6) promoting metabolically significant reaction of hydrogen peroxide degradation. Highly active producer of extracellular catalase – mycelial fungus *Penicillium piceum* BIM F-371 D was derived by the method of adaptive selection to H_2O_2 at laboratory of enzymes, Institute of Microbiology, NAS Belarus. Nutrient medium composition was optimized and cultural parameters of the fungus were defined [1, 2]. Simple and efficient methods of producing catalase enzymes of different purity grades in different preparation forms were developed. It was found that catalase displayed elevated catalytic activity for decomposition of residual H_2O_2 remaining in the wash-water after alkaline-peroxide bleaching of coton woven fabrics. Application of enzyme preparation will allow ruling out the repeated textile washing. The optimum enzyme effect is achieved when it is used at concentration of 3000-4500 U / L at 40 ° C for 10-15 min. Possibility of using the elaborated enzyme product as biocatalyst accelerating degradation of H_2O_2 utilized for disinfection of contact lenses was demonstrated. The obtained results indicate that supply of the enzyme (150-350 U/ml) into peroxide-salt solution results in complete hydrogen peroxide decay by 6 h at temperature 20°C. Efficiency of catalase application as the component of selective agar nutrient media serving for detection and counting of clinically significant pathogenic microorganisms was proven experimentally.

The viability of fungal cultures is known to be maintained by regular transfers to fresh nutrient media. Subculturing may reduce the activity of microbial producers of bioactive substances and one of its shortcomings is possibility of contamination. Most promising methods for long-term conservation are freeze-drying (lyophilization) and cryopreservation.

Aim of this study – investigation of the effects of lyophilization and cryogenic storage on preservation of properties of strain *P. piceum* producer of catalase.

Strain freeze-drying was performed using lyophilization unit Modulyo 4K ("Edwards", UK). The ampoules with mycelium samples and the protective medium – 10% defatted milk upon 30 min centrifugation were kept at -55°C for 3 h and then were left for 2.5 h at room temperature. The vacuum parameters in the course of drying were 5×10^{-2} - 8×10^{-2} mbar. Cryoconservation of the fungus was carried out at -70°C. Skim milk (10 %) was applied as the protectant. Upon storage the culture was defrosted at +37°C in water bath, avoiding overheating.

Micromycete *P. piceum* maintained by freeze-drying and cryoconservation techniques was inoculated after 6 years of storage on the agar media and grown at +25°C on Petri plates with wort agar during 14 days. The impact of preservation methods on catalase synthesis was analyzed in submerged culture on the optimized medium.

As a result of conducted studies it was found that both storage techniques provided a high cell survival rate. Taking into account that lyophilized and cryoconserved cultures retained the capacity to synthesize catalase after 6 years of storage, both methods may be recommended for long-term maintenance of strain-producer.

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DYNAMICS OF PHOTOINDUCED GROWTH ACTIVITY OF EDIBLE AND MEDICINAL MACROMYCETES DURING STORAGE AND RECULTIVATION.

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Earlier we established the possibility of using low-intensity radiation in the visible part of the spectrum to regulate the growth and biosynthetic activity of macromycetes in biotechnological processes of their cultivation. The lack of information about the dynamics of photo-induced activity in the cells of living organisms at the time was the basis for conducting studies of preservation duration on photoinduced activity of edible and medicinal mushrooms seed mycelium during storage and recultivation.

The seed mycelium irradiated in the optimal regime for each strain (230 mJ/cm^2) in the stationary growth phase was stored in the dark at a temperature of +5^{0}C. Sowing on a liquid nutrient medium was made immediately after irradiation and then every 24 hours. An indicator of the seed mycelium activity was the accumulation of biomass for a certain volume of the medium. As a control, a mycelium not exposed to light was used.

It has been established that a significant decrease in the induced activity of the inoculum begins in the first 24 hours of storage in the species *Flamulina velutipes, Hericium erinaceus* and *Inonotus oblicus*. Seed mycelium *Cordiceps militaris, Ganoderma applanatum, Pleurotus ostreatus* and *Lentinus edodes* begin to lose activity 24 hours after irradiation. In *P. ostreatus*, it remains unchanged for the first 24 hours, and in G. lucidum - 48 hours. However, after 72 hours the mycelium inoculum activity in all strains decreased to control level. It will need to be taken into account when bioactivation methods by light of low intensity using in biotechnologies of macromycetes cultivation.

According to the theory of universal mechanisms of photostimulation, the main physical and/or chemical changes caused by light in photoacceptor molecules are accompanied by a cascade of biochemical reactions in cells that do not require further activation by light. However, there is no information about the duration of these processes. Nevertheless, these questions are no less important in the development of high-intensive technologies for cultivation of macromycetes using artificial light than the duration of preservation of photoinduced changes after irradiation.

To obtain an answer to these questions, a series of sequential passages of the photoactivated mycelium was carried out. In *C. militaris, F. velutipes, G. lucidum, L. edodes* and *P. ostreatus* strains activity level did not significantly change after two passages, whereas *G. applanatum, H. erinaceus obliquus* internus and *P. ostreatus* its decrease was observed already after the second subculture. The accumulation of mycelial biomass after inoculation of the fermentation medium with a mycelium obtained after the fourth passage after irradiation did not differ significantly from the control. This allows us to recommend the use of the photoactivated mycelium *C. militaris. F. velutipes, G. lucidum, P. ostreatus* and *L. edode*s for two consecutive inoculations, and for *G. applanatum, H. erinaceus*, and *I. obliquus* for only one.

ISOLATION AND OBTAIN OF PURE YEAST STRAINS FROM THE "TRIFESHTI" VINEYARD FOR PRODUCTION OF DRY WHITE AND RED WINES

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Wine fermentation was traditionally carried out by indigenous yeasts associated with grapes and cellar equipment. Today majority of wine production is based on use of active dried yeast which ensures rapid and reliable fermentation and reduces the risk of sluggish or stuck fermentation and microbial contamination. Most commercial wine yeast strains available today have been selected in the vineyard for oenological traits. Despite the availability of several *Saccharomyces cerevisiae* commercial strains intended for wine production, strains isolated from winery regions are usually more adapted to their own climatic conditions, grapes and also partially responsible for particular characteristics that frequently identify specific wines and regions. The selection of yeasts for winemaking consists of identifying specific cultures, mainly Saccharomyces, which can ferment grape juice effectively and can produce good quality wines.

Thus, the aim of this is to isolate and characterize *S. cerevisiae* strains from *"Trifeshti"* vineyard that could be used as a tool for improving the wine quality and reproducibility of the wines obtained, allowing the creation of strong identity that could facilitate their market insertion.

Isolation and selection of local yeast strains from the vineyard *"Trifeshti"* was carried out by means of obtaining pure cultures from fermenting grape must.

Thus the microbiota of an important winery region *"Trifeshti"* was studied in order to isolate and characterize *Saccharomyces cerevisiae* strains that could be used on wine production.

Samples of grape must was characterized by the following initial indices: sugar concentration – 172/218 g/L, acidity titratable-6,2/6,5 g/L (Aligote/Sauvignon); sugar concentration – 220/230 g/L, acidity titratable-7,7/5,4 g/L (Cabernet-Sauvignon/Merlot).

During fermentation, before withdrawing the samples, the bottles were shaken to thoroughly mix the contents and get all of microbial cells in suspension.

Each of these samples was streaked on 10 Petri dishes containing YPD agar medium (1% yeast extract, 2% peptone, 2% glucose and 1.5% agar) and incubated at 28°C for 3 days to allow colony formation. A total of 47 colonies were selected randomly. Cultures were then stored at 4°C until further analysis.

Identification of these isolates was carried out conventionally using morphological and physiological characterization as described by Burian N. (Practicescaia microbiologhia vinodelia, 2003). From 47 yeast isolated, 32 were identified as *Saccharomyces cerevisiae,* 12 as *Saccharomyces pastorianus* and 3 as *Saccharomyces bayanus*.

Thirty two strains of *Saccharomyces cerevisiae*, isolated from the *"Trifeshti"* vineyard, were tested for: fermentation vigor, ethanol, SO_2, copper and cold resistance, volatile acidity and H_2S production, β-glycosidase activity, foam production, killer character and mode of growth in liquid medium.

Some of them showed advanced technological characteristics indicating that they could be applied on wine production in order to increase the quality and assure the particular wine characteristics of that region. From the results of this investigation we are able to select ten yeast strains for more detailed fermentation trials and possible use as a starter culture in production of typical wines.

In conclusion, this study was useful in obtaining interesting information about the oenological characteristics of *Saccharomyces* tested, which is a necessary first step in strain selection.

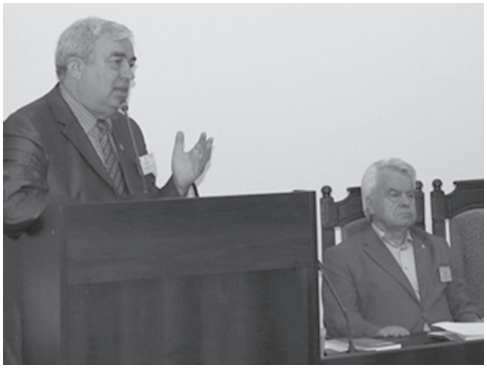
Previous conferences

highlights and photos

International scientific Conference on Microbial BIOTECHNOLOGY (1st edition)

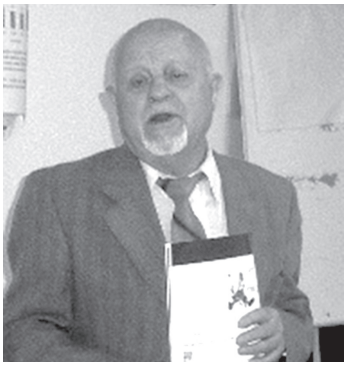
The Conference was held in Chisinau, Republic of Moldova on 6-8 July 2011. The Conference was attended by 284 participants. Participant countries: Romania, Belarus, Ukraine, Poland, Russia, Italy, USA, Turkey, Kazakhstan, Uzbekistan. The main topics for the conference were:

1. Microbiological techniques/biotechnologies for agriculture.
2. Microbiological techniques/ biotechnologies for medicine.
3. Microbiological techniques/ biotechnologies for environment.




International scientific Conference on Microbial BIOTECHNOLOGY (2nd edition)

The Conference was held in Chisinau, Republic of Moldova on 9-10 October 2014. The event was organized by the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova and the Society for Microbiology of Moldova with the support of Federation of European Microbiological Societies. The 2014 edition of the Conference was dedicated to important dates in the history of microbiological science in Moldova: 55th anniversary of the Institute of Microbiology, 20th anniversary of the National Collection of Nonpathogenic Microorganisms and 20th anniversary of the Society for Microbiology of Moldova. The Conference was attended by 202 participants from Moldova, Romania, Ukraine, Italy, Belgium, Belarus, Russia, Bulgaria, and Poland.



international scientific Conference on Microbial BIOTECHNOLOGY (3rd edition)

The Conference was held in Chisinau, Republic of Moldova on 12-13 October 2016. The 2016 edition of the Conference was dedicated to the 70th anniversary of foundation of first research institutions and 55th anniversary of inauguration of the Academy of Sciences of Moldova. The Conference was attended by 250 participants from Moldova, Romania, Ukraine, Italy, Belarus, Russia, Kazakhstan and USA.

Conference topics:

1. Red Biotechnology (Health, Medical, Diagnostics)
2. Yellow Biotechnology (Food Biotechnology, Nutrition Science)
3. Blue Biotechnology (Aquaculture, Costal and Marine Biotech)
4. Green Biotechnology (Agricultural and Environmental Biotechnology)
5. White Biotechnology (Gene-based Bioindustries)
6. Gold Biotechnology (Bioinformatics, Nanobiotechnology)
7. Grey Biotechnology (Classical fermentation and Bioprocess Technology)







Name Index