MINISTRY OF EDUCATION AND RESEARCH OF THE REPUBLIC OF MOLDOVA

IMB INSTITUTE OF MICROBIOLOGY AND BIOTECHNOLOGY

SOCIETY FOR MICROBIOLOGY AND OF MOLDOVA SMM

# International Scientific Conference on Microbial Biotechnology *5th edition*

CHISINAU, MOLDOVA, OCTOBER 12 – 13, 2022

CZU:579+577+60(082)
M 65

**ORGANIZING COMMITTEES:**

Scientific Program Committee:

**Valeriu RUDIC**, professor, Moldova
**Magdalena CARA**, professor, Albania
**Madalin ENACHE**, professor, Romania
**Emmanuel I. SATRATAKIS**, professor, Greece
**Julio SALINAS**, professor, Spain
**Liliana CEPOI**, PhD, Moldova
**Svetlana CODREANU**, PhD, Moldova
**Serghei CORCIMARU**, PhD, Moldova
**Inna RASTIMESINA**, PhD, Moldova

Local Organizing Committee:

**Oleg CHISELITA**, PhD, Moldova
**Vera MISCU**, PhD, Moldova
**Iulia IATCO**, PhD, Moldova
**Ludmila RUDI**, PhD, Moldova
**Tatiana CHIRIAC**, PhD, Moldova
**Olga POSTOLACHI**, PhD, Moldova
**Tamara SIRBU**, PhD, Moldova
**Natalia CHISELITA**, PhD, Moldova
**Steliana CLAPCO**, PhD, Moldova
**Alina BESLIU**, PhD, Moldova

Editing of the collection was carried out with the financial support for the State Program 2020-2023 Projects: ***20.80009.7007.03, 20.80009.5007.05, 20.80009.5107.08, 20.80009.7007.09 and 20.80009.5107.16***, financed by the National Agency for Research and Development.

Descrierea CIP a Camerei Naţionale a Cărţii

**DESCRIEREA CIP A CAMEREI NAȚIONALE A CĂRȚII DIN REPUBLICA MOLDOVA**
**"Microbial Biotechnology", international scientific conference (5 ; 2022 ; Chișinău).** 5th International Scientific Conference on Microbial Biotechnology, 12-13 October 2022, Chisinau / scientific program committee: Valeriu Rudic [et al.] ; local organizing committee: Oleg Chiselita [et al.]. – Chișinău : S. n., 2022 (Artpoligraf). – 120 p. : fig., fot., tab.
Antetit.: Min. of Education and Research of the Rep. of Moldova, Inst. of Microbiology and Biotechnology, Soc. for Microbiology of Moldova. – Referinţe bibliogr. la sfârşitul art. –100 ex.

**ISBN 978-9975-3555-6-8. – ISBN 978-9975-3555-7-5 (PDF).**



Tipărit la Tipografia „Artpoligraf” SRL

*"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.

Starting from 2018, September 17 is an open day for everyone who is passionate about the fascinating world of microscopic organisms. The Institute's laboratories are open to visitors - everyone is welcome! During the visit you will get acquainted with the Collection of non-pathogenic microorganisms and will perform microbiology lab routine activities.

# PROGRAM OVERVIEW

| FIRST DAY<br>WEDNESDAY, OCTOBER 12, 2022 | VENUE:<br>HALL 253, INSTITUTE OF MICROBIOLOGY AND BIOTECHNOLOGY<br>1, ACADEMIEI STR., CHISINAU |
|---|---|
| 09:00 – 09:30 | REGISTRATION |
| 09:30 – 10:00 | OPENING CEREMONY |
| 10:00 – 10:45 | PLENARY SESSION |
| 10:45 – 11:00 | COFFEE BREAK |
| 11:30 – 12:45 | PLENARY SESSION |
| 12:45 – 13:30 | LUNCH |
| 13:30 – 16:30 | THEMATIC SESSION<br>GREEN BIOTECHNOLOGY (AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY) |

| SECOND DAY<br>THURSDAY, OCTOBER 13, 2022 | VENUE:<br>HALL 253, INSTITUTE OF MICROBIOLOGY AND BIOTECHNOLOGY<br>1, ACADEMIEI STR., CHISINAU |
|---|---|
| 08:30 – 09:00 | THEMATIC SESSION<br>GREEN BIOTECHNOLOGY (AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY) |
| 09:00 – 10:00 | THEMATIC SESSION<br>BLUE BIOTECHNOLOGY (AQUACULTURE, COSTAL AND MARINE BIOTECH) |
| 10:00 – 11:15 | THEMATIC SESSION<br>YELLOW BIOTECHNOLOGY (FOOD BIOTECHNOLOGY, NUTRITION SCIENCE) |
| 11:15 – 11:30 | COFFEE BREAK |
| 11:30 – 11:45 | THEMATIC SESSION<br>RED BIOTECHNOLOGY (HEALTH, MEDICAL, DIAGNOSTICS) |
| 11:45 – 12:15 | THEMATIC SESSION<br>GOLD BIOTECHNOLOGY (BIOINFORMATICS, NANOBIOTECHNOLOGY) |
| 12:15 – 13:30 | POSTER SESSION |

# PROGRAMME

| **First day**<br>**WEDNESDAY, October 12, 2022** | | **Venue:**<br>**Hall 253, Institute of Microbiology and Biotechnology**<br>**1, Academiei str., Chisinau** |
|---|---|---|
| **09:00 – 09:30** | **REGISTRATION** | |
| **09:30 – 10:00** | **OPENING CEREMONY** | |
| **10:00 – 12:45** | **PLENARY SESSION** | |
| 10:00 – 10:15 | Cepoi Liliana | IN VIVO ACCUMULATION OF BIOFUNCTIONALIZED AGNPS WITH SPIRULINA |
| 10:15 – 10:30 | Ruginescu Robert | BIOPROSPECTING FOR NOVEL BACTERIAL SOURCES OF SALT-TOLERANT ENZYMES WITH BIOTECHNOLOGICAL APPLICATIONS. |
| 10:30 – 10:45 | Rakhmanov Bakhtiyor | DEVELOPMENT OF GENETIC VECTORS BASED ON ARTEMISININ BIOSYNTHESIS RELATED GENES AND THEIR TRANSFORMATION INTO PLANTS USING AGROBACTERIUM |
| **10:45 – 11:00** | **COFFEE BREAK** | |
| 11:00 – 11:15 | Corcimaru Serghei | LOW DENSITY POLYETHYLENE DEGRADATION BY SOIL MICROORGANISMS |
| 11:15 – 11:30 | Semashko Tatiana | ANALYSIS OF INTERACTION OF SILVER NANOPARTICLES SYNTHETIZED BY PSEUDOMONAS STUTZERI AND TRICHODERMA HARZIANUM WITH OXIDOREDUCTASES |
| 11:30 – 11:45 | Zinicovskaia Inga | EFFECT OF ZINC-CONTAINING WASTEWATER ON *SPIRULINA PLATENSIS* BIOACCUMULATION CAPACITY AND BIOCHEMICAL COMPOSITION |
| 11:45 – 12:00 | Zhukouskaya Liudmila | INFLUENCE OF CULTIVATION CONDITIONS ON THE GROWTH AND FORMATION OF PSEUDOARTROBACTER SCLEROMAE CHOLESTEROL OXIDASE |
| 12:15 – 12:30 | Ungureanu (Negut) Irina | BIOACTIVE GLASS COATINGS SYNTHESIZED BY MAPLE FOR ENHANCED PERFORMANCE OF MEDICAL IMPLANTS |
| 12:30 – 12:45 | Lesnic Evelina | THE CORRELATION BETWEEN THE IMMUNE INDICATORS IN PATIENTS WITH PULMONARY DRUG-SUSCEPTIBLE AND MULTIDRUG-RESISTANT TUBERCULOSIS |
| **12:45 – 13:30** | **LUNCH** | |
| **13:30 – 16:45** | **GREEN BIOTECHNOLOGY (AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY)** | |
| 13:30 – 13:45 | Zakiryaeva Saidakhon | ANTAGONISTIC PROPERTIES OF RHIZOBACTERIA IN RELATION TO WHEAT PHYTOPATOGENES |
| 13:45 – 14:00 | Vorona Valentina | CHARACTERIZATION OF MICROBIAL CONSORTIUM ISOLATED FROM LANDFILL SOIL POLLUTED WITH POLYETHYLENE |
| 14:15 – 14:30 | Stingaci Aurelia | ENTOMOPATHOGENIC BIOPESTICIDES – AN ALTERNATIVE INCREASING THE ADAPTABILITY OF PLANTS TO STRESS IMPACT AND ECO-FRIENDLY SOURCE FOR THE CONTROL OF PESTS |
| 14:30 – 14:45 | Samoilova Anna | EFFECT OF PHAGES ISOLATED FROM DIFFERENT SOURCES AGAINST FIRE BLIGHT PATHOGEN |
| 14:45 – 15:00 | Rastimesina Inna | IDENTIFICATION OF MIXED MICROBIAL CONSORTIA ISOLATED FROM POLYETHYLENE FILMS SURFACE |
| 15:00 – 15:15 | Postolachi Olga | THE EFFECT OF FERTILIZER ON THE ABUNDANCE OF MICROORGANISMS DURING SOIL REMEDIATION |
| 15:15 – 15:30 | Bogdan-Golubi Nina | THE VIABILITY OF BACILLUS, PSEUDOMONAS AND LACTIC ACID BACTERIA STRAINS AFTER 15 YEARS OF STORAGE |
| 15:30 – 15:45 | Codreanu Liviu | CHROMIUM BIOACCUMULATION POTENTIAL OF EDAPHIC CYANOBACTERIUM NOSTOC LINCKIA GROWN ON MULTIMETALLIC SYSTEMS |
| 15:45 – 16:00 | Macari Vasile | THE EFFECTS OF RATION MEDICATION WITH ZooBioR ON SOME PARAMETERS OF MINERAL METABOLISM IN YOUNG CHICKEN |
| 16:00 – 16:15 | Moldovan Anna | GROWTH AND SPORULATION OF BEAUVERIA BASSIANA ON DIFFERENT CULTURE MEDIA |
| 16:15 – 16:30 | Balan Ion | CORRELATIONS OF MICROBIOTA, ENVIRONMENT AND REPRODUCTIVE HEALTH |
| 16:30 – 16:45 | Yushin Nikita | METAL REMOVAL FROM ERBIUM-CONTAINING WASTEWATER USING ARTHOSPIRA PLATENSIS |

| **Second day**<br>**THURSDAY, October 13, 2022** | | **Venue:**<br>**Hall 253, Institute of Microbiology and Biotechnology**<br>**1, Academiei str., Chisinau** |
|---|---|---|
| **08:30 – 09:00** | **GREEN BIOTECHNOLOGY (AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY)** | |
| 08:30 – 08:45 | Enache Madalin | CHARACTERIZATION OF RAW MATERIALS FROM SEVERAL SOURCES FOR OBTAINING BIODEGRADABLE PACKAGING |
| 08:45 – 09:00 | Costin Batrinescu-Moteau | ENDOLITHIC MICROORGANISMS FROM VOLCANIC TUFF |
| **09:00 – 10:00** | **BLUE BIOTECHNOLOGY (AQUACULTURE, COSTAL AND MARINE BIOTECH)** | |
| 09:00 – 09:15 | Sirbu Tamara | ENZYMATIC CAPACITY OF FRESHWATER MICROORGANISMS |
| 09:15 – 09:30 | Moldovan Cristina | STUDY OF THE ENZYMATIC PROPERTIES OF FUNGI IN THE "LA IZVOR" AQUATIC ECOSYSTEM |
| 09:30 – 09:45 | Birsa Maxim | PRESENCE OF ACTINOBACTERIA IN THE AQUATIC ECOSYSTEM OF THE “LA IZVOR” LAKE IN THE CHISINAU |
| 09:45 – 10:00 | Trofim Alina | USE OF THE EXTRACTS OF CYANOBACTERIA CALOTHRIX MARCHICA, NOSTOC HALOPHYLUM AND SPIRULINA PLATENSIS FOR THERAPEUTIC PURPOSES |
| **10:00 – 11:15** | **YELLOW BIOTECHNOLOGY (FOOD BIOTECHNOLOGY, NUTRITION SCIENCE)** | |
| 10:00 – 10:15 | Stici Valentina | USING THE PEARSON CORRELATION BETWEEN PH, TDS AND ORP PARAMETERS WITH MALONDIALDEHYDE AS THE BIOCHEMICAL INDICATOR OF THE PORK QUALITY. |
| 10:15 – 10:30 | Efremova Nadejda | APPLICATION PROSPECT OF AMINOPROTEIC EXTRACTS FROM YEASTS OF WINE SEDIMENTS |
| 10:30 – 10:45 | Chiselita Oleg | ANTOCYANIC EXTRACTS FROM YEASTS WINE WASTE |
| 10:45 – 11:00 | Besliu Alina | CHARACTERIZATION OF MANNOPROTEINS EXTRACTS OBTAINED FROM WINE INDUSTRY WASTE |
| 11:00 – 11:15 | Boistean Alina | ISOLATION, CHARACTERIZATION AND APPLICATION OF ACETIC ACID BACTERIA FROM LOCAL WINE PRODUCTS |
| **11:15 – 11:30** | **COFFEE BREAK** | |
| **11:30 – 11:45** | **RED BIOTECHNOLOGY (HEALTH, MEDICAL, DIAGNOSTICS)** | |
| 11:30 – 11:45 | Rakhmanov Bakhtiyor | BIOTECHNOLOGIES IN ARTEMISININ PRODUCTION |
| **11:45 – 12:15** | **GOLD BIOTECHNOLOGY (BIOINFORMATICS, NANOBIOTECHNOLOGY)** | |
| 11:45 – 12:00 | Valuta Ana | THE ACTION OF GOLD AND SILVER NANOPARTICLES ON PHYCOBILIPROTEIN SYNTHESIS IN RED MICROALGA PORPHYRIDIUM CRUENTUM |
| 12:00 – 12:15 | Condruc Viorica | THE INFLUENCE OF SOME METAL NANOOXIDES ON THE EXOCELLULAR AMYLASE ACTIVITY OF ASPERGILLUS NIGER CNMN FD 06 MYCELIAL FUNGAL STRAIN |
| **12:15 – 13:30** | **POSTER SESSION** | |
| 12:15 – 12:20 | Artiomov Laurenția | ACTINOBACTERIA MICROBIAL COMMUNITY STRUCTURE IN THE AGROECOSYSTEMS SOIL |
| 12:20 – 12:25 | Balacci Sergiu | MODIFICAREA SACULUI VITELIN LA LARVELE DE CRAP ÎN FUNCȚIE DE VARIETATEA TEMPERATURILOR DE MEDIU |
| 12:25 – 12:30 | Chiriac Tatiana | SPIRULINA BIOMASS, CONTAINING SILVER NANOPARTICLES - RAW AND SAFE MATERIAL FOR THE DEVELOPMENT OF MULTIPURPOSE REMEDIES |
| 12:30 – 12:35 | Gutu Nadejda | PATHOGENIC AGENTS OF ACUTE DIARRHEA DISEASE FROM THE ENTEROBACTERIACAEA FAMILY AND THEIR ANTIBIOTIC RESISTANCE |
| 12:35 – 12:40 | Indoitu Diana | PROMISING MICROORGANISMS FOR TREATMENT OF POULTRY PROCESSING WASTEWATER |
| 12:40 – 12:45 | Israyelyan Arevik | ANTIMICROBIAL ACTIVITY OF POLYSACCHARIDES PRODUCED BY LACTIC ACID BACTERIA AGAINST PNEUMONIA PATHOGENS |
| 12:45 – 12:50 | Lungu Andrei | SOME FEATURES OF CULTIVATION OF THE ACTINOBACTERIUM *SACCHAROPOLYSPORA SPINOSA* |
| 12:50 – 12:55 | Malic Alina | EFFICACY AND SAFETY OF THE VIDEO-OBSERVED TREATMENT IN PATIENTS WITH PULMONARY TUBERCULOSIS |
| 12:55 – 13:00 | Moroz Irina | SELENIUM-ENRICHED FODDER YEAST: PRODUCTION AND APPLICATION IN STOCK BREEDING |
| 13:00 – 13:05 | Pantea Valeriana | THE IMPACT OF THE COORDINATIVE COMPOUNDS, THIOSEMICARBASIDE DERIVATES ON THE OXIDATIVE STRESS INDICES IN EX VIVO EXPERIMENTS |
| 13:05 – 13:10 | Sargsyan Anyuta | ROLE OF LACTIC ACID BACTERIA IN ANIMAL FEEDSTUFF |
| 13:10 – 13:15 | Zosim Liliana | ANTIOXIDANT CAPACITY OF THE EXTRACTS OF SPIRULINA BIOMASS CONTAINING PHYCOCYANIN |
| **13:15 – 13:30** | **POSTER SESSION DISCUSSIONS** | |

# Conference

# Proceedings

# PLENARY Session

**_IN VIVO_ ACCUMULATION OF BIOFUNCTIONALIZED AgNPs USING SPIRULINA**

Cepoi L.[1], Zinicovscaia I.[2,3,4], Rudi L.[1], Chiriac T.[1], Peshkova A. [2], Cepoi A.[1], Grozdov D.[2]
[1]*Institute of Microbiology and Biotechnology, Republic of Moldova*
[2]*Department of Nuclear Physics, Joint Institute for Nuclear Research, Russian Federation*
[3]*Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, Romania*
[4]*Institute of Chemistry, Republic of Moldova*
e-mail: cepoililiana67@gmail.com

CZU:57.085:579.6 https://doi.org/10.52757/imb22.01

The 4.7 nm polyethylene glycol-stabilized AgNPs were functionalized with spirulina culture during a cultivation cycle. Experiments with laboratory animals were carried out at the Institute of Physiology and Sanocreatology, the Laboratory of Physiology of Stress, Adaptation and General Sanocreatology, and in the Institute's vivarium. AgNPs were administered to animals of the experimental group at a dose of 1 μg Ag/day/animal. Laboratory animals were divided into several groups: two control groups: rats normally kept without food additives and rats fed with spirulina biomass; and two experimental groups: rats treated with silver nanoparticles and an animal group treated with functionalized silver nanoparticles using spirulina. The experience included an administration period of 28 days followed and a 28 days clearance period. Ag was determined by neutron activation analysis (NAA) in different organs after the animals were sacrificed. In the experimental group treated with AgNPs, the maximum concentrations of 0.145-0.150 μg/g Ag were determined in brain, liver and kidney tissues. The concentration of 0.09 μg/g Ag was determined in the spleen. In this case, no accumulation of silver was found in testicular and ovarian tissues. For AgNP-Spirulina, the highest content of 0.136 μg/g silver was determined in the kidneys. Regardless of the type of nanoparticles, one of the pathways for their elimination is the renal one. In animals treated with functionalized AgNPs, silver was determined in the brain tissue at a concentration of 0.113 μg/g. The difference between the bioaccumulation of AgNPs and AgNP-Spirulina in the brain was insignificant, which indicated the ability of nanoparticles to cross the blood-brain barrier and the lack of the effect of enhancing the bioavailability of AgNPs functionalized with spirulina biomass. The content of 0.07 μg/g Ag was determined in the spleen, which corresponds to the value determined in the liver. The silver content determined as a result of the administration of functionalized AgNP-Spirulina in the liver was twice lower than that of silver in the non-functionalized nanoparticles accumulated in the organ. If this was not an increase in the bioavailability of silver nanoparticles, then the relatively low value of AgNP-Spirulina in the liver could be the result of their rapid metabolism. Silver nanoparticle content of 0.085 μg/g was determined in testicular tissue as a result of the administration of functionalized AgNP-Spirulina. This result may be due to the affinity of the tissue in division for some components of the spirulina biomass. This property of AgNP-Spirulina can be further analyzed for practical purposes, as well as the effect of the lack of functionalized nanoparticles in ovarian tissue. Silver was determined in the organs of animals subject to a clearance period of 28 days. In the experimental variant of AgNPs administration, at the expiration of the recovery time, silver was determined in the brain and liver. Thus, in the brain tissue, the Ag content was 0.097 μg/g, which was 2/3 of Ag concentration established as a result of the administration of nanoparticles for 28 days. Then, 24% of the amount of accumulated silver remained in the liver. For AgNP-Spirulina, the absence of nanoparticles in tissues (liver, spleen, kidneys, testicles) was found, except for the brain. The content of silver nanoparticles in brain tissue taken from the animals subject to clearance period was 0.093 μg/g. The obtained result confirms the impossibility of the return path across the blood-brain barrier.

It has been established that AgNPs functionalized with spirulina acquire new biological properties, different from those of non-functionalized AgNPs, and consequently new areas of application.

*The scientific results were obtained within the project 20.80009.5007.05 „Biofunctionalized metal nanoparticles - obtaining using cyanobacteria and microalgae" funded by NARD, Republic of Moldova.*

# BIOPROSPECTING FOR NOVEL BACTERIAL SOURCES OF SALT-TOLERANT ENZYMES WITH BIOTECHNOLOGICAL APPLICATIONS

Ruginescu R.[1], Gomoiu I.[1], Neagu S.[1], Cojoc R.[1], Lucaci I.[1],
Batrinescu-Moteau C.[1], Enache M.[1]
[1]*Institute of Biology Bucharest, Romanian Academy, Bucharest, Romania*
e-mail: robert.ruginescu@ibiol.ro

CZU:606:579.6 https://doi.org/10.52757/imb22.02

Extracellular enzymes produced by halophilic and halotolerant microorganisms have evolved to retain structural stability and catalytic activity over a wide range of salinities and thus they could be useful in numerous industrial and environmental applications where high salt concentrations would otherwise inhibit enzymatic transformations.

Considering the biotechnological importance of salt-tolerant enzymes, the growing demand for these molecules on the global market and the current need for more efficient producers of such biocatalysts, the aim of the present study was to isolate and identify novel strains of halophilic and halotolerant microorganisms able of synthesizing extracellular hydrolases. In order to achieve this goal, five under-/uninvestigated salt lakes in Romania (i.e., Lake Amara, Lake Balta Alba, Lake Caineni-Bai, Movila Miresii Salt Lake and Braila Salt Lake) and the littoral zone of the Black Sea were sampled and subjected to bioprospecting studies. A total of 151 strains (138 bacteria and 13 archaea) were isolated and identified based on 16S rRNA gene sequencing. The bacterial strains belonged to the classes *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacilli*, *Flavobacteriia* and *Actinobacteria*, while the archaeal strains belonged to the class *Halobacteria*.

The screening for hydrolytic enzymes showed that most of the strains were able to produce single or combined hydrolytic activities (i.e., protease, lipase, amylase, cellulase, xylanase and pectinase). The enzymes produced by three selected strains belonging to the genus *Bacillus* were active over a wide range of salt concentrations, temperatures and pH values. Due to such functional properties, these hydrolases could be suitable in various applications that require harsh physicochemical conditions.

# LOW DENSITY POLYETHYLENE DEGRADATION BY SOIL MICROORGANISMS

Corcimaru S.[1], Mereniuc L.[1], Sîtnic F.[1], Rastimesina I.[1], Gutul T.[2]
[1]*The Institute of Microbiology and Biotechnology, The Republic of Moldova*
[2]*Ghitu Institute of Electronic Engineering and Nanotechnologies*
e-mail: sergheicorcimaru@hotmail.com

CZU:632.15 https://doi.org/10.52757/imb22.03

## Introduction

In the Republic of Moldova there is an acute problem of environmental pollution by plastics, such as the low-density polyethylene (LDPE). The share of plastics in the worldwide waste volume is about 10-30%, and the rate of just polyethylene waste accumulation in the environment is 25 million tons/year [1]. From 2019 in the Republic of Moldova it has been mandatory to recycle at least 10% of plastic packaging, and from 2025 this share will increase to 20% [2]. However, there currently are no efficient technologies that could be used for the recycling/processing of low-density polyethylene (LDPE) – the basic material of the most widespread type of packaging, including single-use bags [3]. Although in the Republic of Moldova, according to the legislation [4-5], these bags are to be gradually withdrawn from circulation, nevertheless for a long time LDPE will remain among the main persistent environmental pollutants. Due to the lack of chemical and physical methods for efficient and sustainable degradation of LDPE, in the last decade attention has been directed toward development of microbiological biodegradation technologies [1, 3, 6-11], including with application of nanomaterials [12-13]. These technologies are based on the microorganisms, that can use LDPE as a source of carbon and/or energy [8, 11], and on nanomaterials that can stimulate the LDPE biodegradation. The efficiency of these technologies, among other things, depends on the identification and isolation of these microorganisms, on the elaboration of the most efficient nanomaterials, and on considering all other possible factors that can stimulate the biodegradation of LDPE. Potentially, the LDPE degrading microorganisms can be found in soils with high microbial biodiversity and/or in soils from the territories subjected to long-term pollution by plastics.

The purpose of this work was to estimate the LDPE biodegradation rates and the possibilities of their acceleration in soil from two contrasting territories from the Republic of Moldova – a virgin forest soil, and in a landfill with plastics and other contaminants.

## Materials and methods

The LDPE biodegradation experiments were conducted under laboratory conditions and based on soil samples collected from two contrasting territories in the Republic of Moldova. The first soil sample was collected from a forest in the Orhei district. And the second – from a landfill near the locality of Slobozia-Dusca, the Criuleni district. Soil samples were collected in the spring of 2021 from a depth of 0-10 cm; passed through a 2 mm sieve and plant material, stones and visible organisms removed manually; adjusted to 40% water holding capacity and pre-incubated for 10 days at 25ºC in the dark, in aerated plastic bags (to prevent accumulation of $CO_2$) with periodic adjustment of moisture (to prevent drying). The soil organic matter content (SOM) was determined by dichromate oxidation followed by back titration of the excess dichromate [14]. The soil microbial biomass (SMB) was determined by substrate-induced respiration [15]. The basal respiration (BR) was determined by measuring $CO_2$ emission from soil by the Li-850 IRGA [16]. Metabolic quotient ($qCO_2$) was calculated as BR expressed per mg of SMB carbon.

The comparison of the properties of the selected soils indicated the presence of a relatively high ecological stress in the soil of the landfill – the microbial biomass was 27 times lower, and the metabolic coefficient (the indicator of the state of ecological stress for soil microorganisms [17]) was 99 times higher than in the forest soil (tab. 1).

**Table 1. The soil parameters**

| N. | Territory | SOM, % | pH | W, % | SMB, µg C/g | BR, µg C-$CO_2$/g/h | $qCO_2$, C-$CO_2$/$mg_{biomass}$/h |
|---|---|---|---|---|---|---|---|
| 1. | The forest in the Orhei district | 6,71±0,02 | 6,9 | 28,1 | 914,2±22,4 | 0,39±0,03 | 0,42±0,03 |
| 2. | The landfill at the locality of Slobozia-Dusca | 4,88±0,03 | 7,9 | 19,7 | 33,8±8,3 | 1,42±0,09 | 41,96±3,05 |

Note: SOM – the soil organic matter content, W – soil moisture content at 40% of the water holding capacity, SMB – the soil microbial biomass, BR – the basal (soil) respiration, $qCO_2$ – the metabolic quotient. The statistics is presented via the confidence interval at P=0,95.

The biodegradation rates were studied first within 34-36-day (34 days for the forest soil and 36 for the landfill soil) incubational experiments with introduction of LDPE, treated and untreated by different nanocomposites, into 130 g of wet soil kept in 250 mL Erlenmeyer flasks (in 3-4 replica per variant). LDPE was used in the form of two film strips cut longitudinally (190x10 mm) and transversally (210x10 mm) from a standard LDPE sheet (210x190 mm). After the end of the first incubation period soil samples were taken for measuring SMB, BR, and $qCO_2$. Then, the second incubation was launch by adding glucose (0.5%) into soil. This incubation lasted for 41 days. In the end of it the same microbial parameters as well as SOM and LDPE weight losses were measured. During the incubations the flasks were kept opened at the room temperature, in the dark, with periodic adjustment of moisture.

The nanocomposites used in the study were based on iron oxide doped with cobalt or magnesium and modified by hydrophilic polymers – polyvinylpyrrolidone (PVP) or polyethylene glycol (PEG). The $CoFe_2O_4$/PEG, $MgFe_2O_4$/PEG, $CoFe_2O_4$/PVP, and $MgFe_2O_4$/PVP nanocomposites were obtained via hydrothermal synthesis from iron salts, cobalt, and magnesium at the temperature of 150° C, in water-alcohol medium, using PEG or PVP as stabilizers. The nanomaterials were in the form of a black powder. According to the confocal microscopy and SEM, the dimensions of the nanomaterials were 50-120 nm. The nanocomposites were identified by the X-ray diffraction, IR spectroscopy, atomic absorption spectroscopy, and thermogravimetric analysis.

Hydrocolloidal suspensions of the nanocomposites in the concentrations of 20 mg/L (min) and 100 mg/L (max) were obtained by treating the suspensions with ultrasound for 3 mins at 50 kHz. LDPE strips were placed into Erlenmeyer flasks with these suspensions and agitated for 1 hour at 200 rpm. Then the strips were placed on filter paper and dried for 3-5 days at the room temperature. Before introduction into soil the strips were exposed to UV radiation for 1 hour 2 times. The study included the following variants of nanocomposites: $CoFe_2O_4/PVP_{min}$, $CoFe_2O_4/PVP_{max}$, $CoFe_2O_4/PEG_{min}$, $CoFe_2O_4/PEG_{max}$, $MgFe_2O_4/PVP_{min}$, $MgFe_2O_4/PVP_{max}$, $MgFe_2O_4/PEG_{min}$, and $MgFe_2O_4/PEG_{max}$.

The statistical analysis was done with the Ms Excel 365 software.

**The Results**

The introduction of untreated LDPE (without the nanocomposites) into each soil did not change the soil microbial activity as compared to the corresponding controls within 34-36 incubation days (tab. 2). The treatment of LDPE with the nanocomposites resulted in several statistically significant changes (tab. 2). For example, within the variants of the forest soil there were observed 9% increases of SMB for $CoFe_2O_4/PVP_{max}$ and $CoFe_2O_4/PEG_{max}$, a 5% decrease of SMB for $MgFe_2O_4/PEG_{max}$, and 14%, 18%, and 20% decreases of $qCO_2$ for $MgFe_2O_4/PEG_{min}$, $MgFe_2O_4/PVP_{max}$, and $CoFe_2O_4/PEG_{max}$ respectively. All these changes were statistically significant comparing to the control variant. Within the variants of the landfill soil $CoFe_2O_4/PEG_{max}$ caused 6% and 8% decreases in SMB comparing to the untreated LDPE and control variants respectively. Also, in the case of $MgFe_2O_4/PVP_{max}$ there was a 9% increase in SMB (comparing to the LDPE variant) that coincided with 21-22% decreases in $qCO_2$ comparing to the control and LDPE variants respectively.

The 41-day incubation with glucose significantly decreased the SOM content in all variants (tab. 3). In the forest soil these decreases were similar in their magnitude and, consequently, did not result in significant differences between the variants including the control. The only exception was SOM in the case of $CoFe_2O_4/PVP_{max}$ that was 4% smaller than in the control. In the landfill soil the glucose introduction resulted in different SOM decreases depending on the case. The control lost 5% of the initial SOM, while

the LDPE, $CoFe_2O_4/PEG_{min}$, $CoFe_2O_4/PEG_{max}$, $MgFe_2O_4/PVP_{min}$ and $MgFe_2O_4/PEG_{min}$ variants lost even more and had 4-7% less of SOM than the control (by the end of the incubation with glucose). SOM in the rest 4 nanocomposite variants with the landfill soil was not different statistically from the control.

**Table 2. The soil parameters after the incubation with LDPE, treated and untreated by nanocomposites.**

| N. | Soil | Variant | LDPE load, g/kg sol | SMB, μg C/g | BR, μg C-$CO_2$/g/h | $qCO_2$, C-$CO_2/mg_{biomass}$/h |
|---|---|---|---|---|---|---|
| 1. | The forest soil | Control | - | 352,08±6,81 | 0,26±0,01 | 0,74±0,02 |
| 2. | | LDPE | 1,37 | 360,17±18,25 | 0,25±0,02 | 0,69±0,09 |
| 3. | | $CoFe_2O_4/PVP_{min}$ | | 344.28±1.92 | 0.26±0. 01 | 0.75±0.02 |
| 4. | | $CoFe_2O_4/PVP_{max}$ | | 383.53±12.09 | 0.26±0.01 | 0.68±0.04 |
| 5. | | $CoFe_2O_4/PEG_{min}$ | | 368.83±12.11 | 0.24±0.01 | 0.66±0.04 |
| 6. | | $CoFe_2O_4/PEG_{max}$ | | 382.91±16.62 | 0.23±0.01 | 0.60±0.06 |
| 7. | | $MgFe_2O_4/PVP_{min}$ | | 339.56±10.31 | 0.24±0.01 | 0.71±0.03 |
| 8. | | $MgFe_2O_4/PVP_{max}$ | | 350.75±5.00 | 0.21±0.02 | 0.61±0.06 |
| 9. | | $MgFe_2O_4/PEG_{min}$ | | 356.03±2.90 | 0.23±0.01 | 0.64±0.01 |
| 10. | | $MgFe_2O_4/PEG_{max}$ | | 333.10±4.26 | 0.24±0.01 | 0.72±0.03 |
| 11. | The landfill soil | Control | - | 35,92±1,71 | 0,50±0,09 | 7,15±0,75 |
| 12. | | LDPE | 1,27 | 35,09±0,91 | 0,69±0,03 | 7,04±0,57 |
| 13. | | $CoFe_2O_4/PVP_{min}$ | | 34.63±0.78 | 0.52±0.10 | 7.46±0.10 |
| 14. | | $CoFe_2O_4/PVP_{max}$ | | 33.95±0.82 | 0.63±0.18 | 7.71±0.37 |
| 15. | | $CoFe_2O_4/PEG_{min}$ | | 34.90±1.30 | 0.62±0.14 | 6.96±0.55 |
| 16. | | $CoFe_2O_4/PEG_{max}$ | | 33.16±0.78 | 0.59±0.02 | 6.87±0.31 |
| 17. | | $MgFe_2O_4/PVP_{min}$ | | 35.21±4.35 | 0.45±0.04 | 6.88±0.79 |
| 18. | | $MgFe_2O_4/PVP_{max}$ | | 38.36±1.88 | 0.57±0.06 | 5.56±0.73 |
| 19. | | $MgFe_2O_4/PEG_{min}$ | | 34.74±0.66 | 0.45±0.05 | 6.52±0.29 |
| 20. | | $MgFe_2O_4/PEG_{max}$ | | 33.69±2.56 | 0.47±0.08 | 7.11±0.37 |

Note: Control – the variant with untreated soil, LDPE – introduction into the soil of untreated low-density polyethylene, $Co/MgF_2O_4$ – introduction into the soil of LDPE treated by the nanocomposites stabilized with polyvinylpyrrolidone (PVP) or polyethylene glycol (PVP) and used in minimal (min) or maximal (max) concentrations. The rest of abbreviations are the same as in tab. 1. The duration of the incubation was 34 days for the forest soil and 36 for the landfill soil. The statistics are shown via the confidence interval at P=0,95.

The glucose introduction into the soil had different effects on the microbial parameters by the end of the incubation (tab. 3). In the forest soil there were observed statistically significant SMB changes in 4 cases out of 10: the LDPE and $MgFe_2O_4/PEG_{min}$ variants had a 6% increase each, comparing to the control, while the $CoFe_2O_4/PVP_{max}$ and $CoFe_2O_4/PEG_{min}$ variants had a 6% decrease each, comparing to the LDPE variant. In the landfill soil there were no statistical differences between the control and LDPE variants, while in all cases with nanocomposites SMB was always statistically higher (from +38% to +60%) than in the control, and $qCO_2$ was always statistically lower than both in the control (from -53% to -67%) and LDPE (from -47% to -62%) variants.

The biggest SMB was observed in the case of $MgFe_2O_4/PVP_{max}$, and the smallest $qCO_2$ – in $MgFe_2O_4/PVP_{min}$. The measurements of the LDPE degradation by the end of incubation with glucose revealed 0,5-2,5% decreases in LDPE mass depending on the soil and the applied nanocomposite (tab. 4).

Within the forest soil variants, the highest degradation was in the LDPE variant. The tested nanocomposites were not able to stimulate the LDPE biodegradation in this soil. In all but one nanocomposite cases the LDPE weight losses were statistically not different from the one in the variant with untreated LDPE. The loss in the $CoFe_2O_4/PVP_{min}$ variant was 46.5 times smaller. Within the landfill soil the smallest degradation rate was in the LDPE variant, and the application of nanocomposites was able to increase it statistically by 3.6-4.3 times in four variants out of 8 ($CoFe_2O_4/PVP_{min}$, $CoFe_2O_4/PEG_{min}$, $MgFe_2O_4/PEG_{min}$, and $MgFe_2O_4/PEG_{max}$).

**Table 3. The soil parameters after the 41-day incubation with glucose.**

| N. | Soil | Variant | SOM, % | SMB, µg C/g | BR, µg C-$CO_2$/g/h | $qCO_2$, C-$CO_2$/$mg_{biomass}$/h |
|---|---|---|---|---|---|---|
| 1. | The forest soil | Control | 6,21±0,13 | 709,56±16,59 | 0,99±0,14 | 1,40±0,21 |
| 2. | | LDPE | 6,18±0,10 | 752,57±9,73 | 1,02±0,05 | 1,26±0,18 |
| 3. | | $CoFe_2O_4$/$PVP_{min}$ | 5.98±0.18 | 743.52±78.17 | 0.75±0.15 | 1.02±0.31 |
| 4. | | $CoFe_2O_4$/$PVP_{max}$ | 5.95±0.12 | 705.21±12.67 | 0.89±0.03 | 1.27±0.04 |
| 5. | | $CoFe_2O_4$/$PEG_{min}$ | 6.05±0.12 | 704.69±21.48 | 0.92±0.17 | 1.31±0.28 |
| 6. | | $CoFe_2O_4$/$PEG_{max}$ | 6.05±0.12 | 738.99±35.29 | 0.82±0.08 | 1.11±0.15 |
| 7. | | $MgFe_2O_4$/$PVP_{min}$ | 5.98±0.14 | 716.88±49.81 | 0.82±0.15 | 1.14±0.20 |
| 8. | | $MgFe_2O_4$/$PVP_{max}$ | 6.09±0.07 | 724.32±20.72 | 0.98±0.05 | 1.35±0.07 |
| 9. | | $MgFe_2O_4$/$PEG_{min}$ | 6.05±0.12 | 751.55±12.50 | 0.76±0.13 | 1.01±0.18 |
| 10. | | $MgFe_2O_4$/$PEG_{max}$ | 6.40±0.14 | 751.33±37.09 | 0.84±0.35 | 1.11±0.45 |
| 11. | The landfill soil | Control | 4,62±0,05 | 459,41±40,65 | 2,39±0,17 | 5,26±0,93 |
| 12. | | LDPE | 4,36±0,05 | 523,97±90,51 | 2,41±0,68 | 4,66±1,72 |
| 13. | | $CoFe_2O_4$/$PVP_{min}$ | 4.53±0.03 | 652.94±120.76 | 1.24±0.65 | 1.84±0.62 |
| 14. | | $CoFe_2O_4$/$PVP_{max}$ | 4.79±0.24 | 690.92±119.89 | 1.55±0.21 | 2.26±0.28 |
| 15. | | $CoFe_2O_4$/$PEG_{min}$ | 4.28±0.07 | 635.40±85.09 | 1.57±0.11 | 2.49±0.34 |
| 16. | | $CoFe_2O_4$/$PEG_{max}$ | 4.31±0.12 | 665.40±90.08 | 1.40±0.11 | 2.13±0.31 |
| 17. | | $MgFe_2O_4$/$PVP_{min}$ | 4.28±0.09 | 723.64±79.70 | 1.29±0.36 | 1.76±0.33 |
| 18. | | $MgFe_2O_4$/$PVP_{max}$ | 4.47±0.27 | 733.02±91.70 | 1.33±0.47 | 1.85±0.77 |
| 19. | | $MgFe_2O_4$/$PEG_{min}$ | 4.43±0.09 | 725.19±65.22 | 1.37±0.39 | 1.90±0.57 |
| 20. | | $MgFe_2O_4$/$PEG_{max}$ | 4.53±0.09 | 714.70±41.07 | 1.36±0.22 | 1.90±0.30 |

Note: See the notes for tab. 1-2.

**Table 4. The LDPE degradation**

| Nr. | Soil | Variant | Applied LDPE weight, g | LDPE weight loss, mg | LDPE degradation, % |
|---|---|---|---|---|---|
| 1. | The forest soil | LDPE | 0,134±0,005 | 3,100±1,978 | 2,299±1,408 |
| 2. | | $CoFe_2O_4$/$PVP_{min}$ | 0.125±0.003 | 0.067±0.131 | 0.05±0.11 |
| 3. | | $CoFe_2O_4$/$PVP_{max}$ | 0.129±0.006 | 3.283±3.163 | 2.54±2.40 |
| 4. | | $CoFe_2O_4$/$PEG_{min}$ | 0.133±0.002 | 2.800±3.941 | 2.11±2.99 |
| 5. | | $CoFe_2O_4$/$PEG_{max}$ | 0.127±0.009 | 1.433±1.641 | 1.08±1.23 |
| 6. | | $MgFe_2O_4$/$PVP_{min}$ | 0.127±0.006 | 1.167±2.287 | 0.88±1.72 |
| 7. | | $MgFe_2O_4$/$PVP_{max}$ | 0.123±0.005 | 0.500±0.837 | 0.39±0.66 |
| 8. | | $MgFe_2O_4$/$PEG_{min}$ | 0.125±0.001 | 1.300±0.765 | 1.03±0.60 |
| 9. | | $MgFe_2O_4$/$PEG_{max}$ | 0.127±0.002 | 3.067±1.139 | 2.40±0.86 |
| 10. | The landfill soil | LDPE | 0,136±0,004 | 0,800±0,564 | 0,582±0,392 |
| 11. | | $CoFe_2O_4$/$PVP_{min}$ | 0.130±0.005 | 3.050±0.889 | 2.34±0.66 |
| 12. | | $CoFe_2O_4$/$PVP_{max}$ | 0.129±0.006 | 1.917±1.443 | 1.46±1.05 |
| 13. | | $CoFe_2O_4$/$PEG_{min}$ | 0.136±0.006 | 3.350±0.493 | 2.46±0.26 |
| 14. | | $CoFe_2O_4$/$PEG_{max}$ | 0.131±0.005 | 2.133±2.417 | 1.61±1.79 |
| 15. | | $MgFe_2O_4$/$PVP_{min}$ | 0.130±0.002 | 1.733±1.077 | 1.33±0.82 |
| 16. | | $MgFe_2O_4$/$PVP_{max}$ | 0.135±0.005 | 1.950±1.318 | 1.43±0.92 |
| 17. | | $MgFe_2O_4$/$PEG_{min}$ | 0.133±0.007 | 3.350±1.225 | 2.50±0.77 |
| 18. | | $MgFe_2O_4$/$PEG_{max}$ | 0.135±0.005 | 2.833±1.454 | 2.09±1.07 |

Note: See the notes for tab. 1-2.

**Discussion**

The absence of statistically significant changes in the microbial parameters after the initial soil incubation with the introduced untreated LDPE showed that the microorganisms of the two tested soils could hardly use LDPE as a source of carbon and/or energy. This agrees well with the general knowledge that polyethylene is extremely recalcitrant to degradation by microorganisms, and that under usual soil conditions it takes years for the biodegradation to come close to 0.5% [18]. The negative implications for the environment in this case are obvious. However, the obtained results also demonstrated that soil amendments with glucose and LDPE treatment by iron oxide-based nanocomposites may considerably accelerate LDPE biodegradation.

Glucose is a source of easily degradable organic carbon that, being introduced into soil, can stimulate the mineralization of the recalcitrant forms of carbon within SOM [19]. This phenomenon is known among the so-called soil priming effects and, in our case, its presence was manifested in the statistically significant SOM decreases in absolutely all variants on day 41 after the glucose introduction. Although not usually used for stimulating LDPE biodegradation, priming effects were suggested as a strategy for degradation of soil organic pollutants [20]. According to our results, the glucose induced priming effects are the best explanation for the increased LDPE weight losses in at least some variants. For example, within the forest soil the second-best degradation rate was observed in the LDPE variant, which also had the highest SMB. The statistically significant 6% increase of SMB in this variant comparing to the control could not result from the initial ability of the soil microorganisms to actively degrade LDPE since, as it was already mentioned, the introduction of LDPE did not cause any SMB stimulation before the incubation with glucose. The fact that by the end of that incubation the SOM content was practically identical to the control one implied that this increase could not result from higher rates of SOM degradation too. Thus, there is good evidence that the glucose induced priming effects stood behind both observed phenomena – the increased microbial biomass and the high LDPE degradation rate. To the best of our knowledge this is one of the first demonstrations of the possibility of stimulating LDPE biodegradation in soil via glucose amendments.

Iron oxide nanoparticles and nanocomposites (as the ones used in this study) are known for their ability to stimulate biodegradation of LDPE. They, among other things, may contribute to capturing $O_2$ in the LDPE hydrocarbon chain and through that stimulate its further bioactive hydrolysis [12]. The obtained results showed that the tested nanocomposites could indeed substantially increase the LDPE degradation rate, but their influence depended on the soil and on whether the soil was amended with glucose or not. The nanocomposites were ineffective in the forest soil conditions, and in one case they even significantly inhibited the LDPE biodegradation. In the landfill soil variants, judging by the absence of any positive impact on SMB comparing to the control, they were quite inefficient too. But the things changed radically after the landfill soil was amended with glucose. All the nanocomposite variants had significantly better microbial parameters comparing to the control, and in 4 out of 8 cases the LDPE degradation rate was up to 4.3 times higher than in the variant with the untreated LDPE. The facts that by the end of the incubation with glucose the biodegradation was much smaller in the variant with the untreated LDPE, and that the microbial biomass of the latter was not statistically different from the control, showed that neither the glucose amendments nor nanocomposites could efficiently stimulate the LDPE degradation by themselves, and that their efficiency became visible only when they were used together. Again, to the best of our knowledge, this is the first-time demonstration that glucose amendments can stimulate the ability of iron oxide-based nanocomposites to stimulate the LDPE biodegradation in soil.

**Bibliography:**

1. Shalini R., Sasikumar C. Biodegradation of low-density polythene materials using microbial consortium – an overview. International Journal of Pharmaceutical and Chemical Sciences, 4 (4), 2015, p.507-514.
2. Lege privind deşeurile Nr. 209 din 29.07.2016. Publicat: 23.12.2016 în Monitorul Oficial Nr. 459-471, art Nr: 916. http://lex.justice.md/md/368030/ (vizitat 1.10.2019).
3. Begum M.A.; Varalakshmi B.; Umamagheswari K. Biodegradation of Polythene Bag using Bacteria Isolated from Soil (2015) International Journal of Current Microbiology and Applied Sciences. 4(11), 2015, p. 674-680.
4. Lege cu privire la comerţul interior Nr. 231 din 23.09.2010. Publicat: 19.08.2016 în Monitorul Oficial Nr. 265-276, art Nr: 571. http://lex.justice.md/md/336378/ (vizitat 1.10.2019).

5. Lege pentru completarea Legii nr. 231/2010 cu privire la comerţul interior Nr. 134 din 07.07.2017. Publicat: 28.07.2017 în Monitorul Oficial Nr. 265-273, art Nr: 438. http://lex.justice.md/md/371663/ (vizitat 1.10.2019).
6. Das M. P., Kumar S. An approach to low-density polyethylene biodegradation by Bacillus amyloliquefaciens. 3 Biotech., 5, 2015, p.81-86.
7. Contat-Rodrigo I.L., Ribes Greus A. Biodegradation studies on LDPE filled with biodegradable additives: morphological changes. J Appl Polym Sci, 83, 2002, p.1683-1691.
8. Aswale P., Ade A. Assessment of the biodegradation of polythene. Bioinfolet, 5, 2008, p.239.
9. Tribedi P., Sil A.K. Low-density polyethylene degradation by Pseudomonas sp. AKS2 biofilm. Environ Sci Pollut Res, 20, 2013, p.4146-4153.
10. Koutny M., Sancelme M., Dabin C., Pichon N., Delort A.M. et al. Acquired biodegradability of polyethylenes containing prooxidant additives. Polym Degrad Stab, 91, 2006, p.1495-1503.
11. Rajendran S.D., et al. (2015). The role of microbes in plastic degradation. doi: 10.1201/b19243-13.
12. Bhatia et al., (2013). Implicating Nanoparticles as Potential Biodegradation Enhancers: A Review. Journal of Nanomedicine and Nanotechnology, 4:4. DOI: 10.4172/2157- 7439.1000175.
13. Kumari, G.; Tiwari, A.; Yadav, M. (2017). LDPE-biodegradation using microbial consortium by the incorporation of Cobalt Ferrite Nanoparticle as the enhancer for biodegradation. International Journal of Advance Engineering and Research Development, V. 4, Issue 6., 794-800. p-ISSN (P): 2348-6406.
14. Аринушкина Е. В. Руководство по химическому анализу почв. 1970. МГУ.
15. Корчмару С. Оценка метода субстрат-индуцированного дыхания (для определения почвенной микробной биомассы) на примере почв Молдовы. Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţe biologice, chimice şi agricole, n. 1 (307), 2009, p. 134-142.
16. Dossaet G. G. O., et al. Correct calculation of CO2 efflux using a closed-chamber linked to a non-dispersive infrared gas analyzer. Methods in Ecology and Evolution, 6, 2015, p.1435–1442.
17. Wardle D.A., Ghani A. A critique of the microbial metabolic quotient (qCO2) as a bioindicator of disturbance and ecosystem development. Soil Biology & Biochemistry. 1995. 27, p. 1601-1610.
18. Krueger, M. C., Harms, H., and Schlosser, D. (2015). Prospects for microbiological solutions to environmental pollution with plastics. Appl. Microbiol. Biotechnol. 99, 8857–8874. doi: 10.1007/s00253-015-6879-4.
19. Kuzyakov Y. Priming effects: Interactions between living and dead organic matter. Soil Biology and Biochemistry. 2010, 42, 1363-1371.
20. Di Lonardo D.P. The microbial side of soil priming effects. PhD thesis, Wageningen University, Wageningen, the Netherlands (2019) ISBN: 978-94-6343-588-8 DOI: https://doi.org/10.18174/470122.

*Acknowledgements. The presented results were obtained within research project 20.80009.7007.03 Microbial Tools for Degradation of Non-Recyclable Plastics Waste of the State Program for 2020-2023 financed by the National Agency for Research and Development of the Republic of Moldova.*

**ANALYSIS OF INTERACTION OF SILVER NANOPARTICLES SYNTHESIZED BY *PSEUDOMONAS STUTZERI* AND *TRICHODERMA HARZIANUM* WITH OXIDOREDUCTASES**

Semashko T.[1], Zhukouskaya L.[1], Zaynitdinova L.[2], Lazutin N.[2]
[1]*Institute of Microbiology of the National Academy of Sciences of Belarus, Belarus*
[2]*Institute of Microbiology of the Uzbekistan Academy of Sciences, Uzbekistan*
e-mail: tsemashko@mbio.bas-net.by

CZU:579.6 https://doi.org/10.52757/imb22.04

Recent studies of new cost-effective and environmentally friendly methods for obtaining metal nanoparticles are of great interest. In this regard, particular attention is given to production of nanoparticles by biological methods using cultures of microorganisms. A very important feature of this method is the possibility of obtaining stable nanoparticles of various shapes and sizes including particles with unusual properties which offers great opportunities for their usage in various fields of industry and agriculture.

The purpose of this work is to obtain silver nanoparticles (NPs) using *Pseudomonas stutzeri* and *Trichoderma harzianum* and analyze the effect on the activity of cholesterol oxidases and glucose oxidases.

At the Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan, solutions of NPs synthesized by *T. harzianum* (passed through the filter) and NPs synthesized by *P. stutzeri* were prepared in culture liquid (CL) and separated from CL by centrifugation. An analysis of the UV spectra of NP solutions showed that absorption maxima are observed in the wavelength range of 190–230 nm, 250–280 nm, and 380–420 nm, and the absorption band in the form of the shoulder at 302 nm, corresponding to silver NPs and silver ions, which is typical for both clusters and reduced silver NPs.

Employees of the Institute of Microbiology of the National Academy of Sciences of Belarus obtained enzyme preparations of cholesterol oxidase (ChO) from *Penicillium kapuscinskii* and *P. roquefortii*, as well as glucose oxidase (GOX) from *P. adametzii*. To analyze the effect of NPs synthesized by microorganisms on the activity of enzymes the initial components were used in the following ratios 1:50, 1:100, 1:200, 1:500, 1:1000.

As a result of the experiments, it was shown that NPs in various concentrations have both stimulating and depressing effects on the activity of these enzymes. It has been established that NPs in conjunction with *P. stutzeri* cells have the maximum stimulating effect on the activity of ChO *P. kapuscinskii*. In the ratio of NP/enzyme 1:100, an increase in the activity of ChO by 2.16 times was observed. Usage of NPs synthesized by *T. harzianum* in a ratio of 1:100 and 1:200, the activity of ChO increased insignificantly by 1.33 and 1.16 times respectively. Inhibition of the catalytic activity of ChO *P. kapuscinskii* by 1.2–3.0 times was observed when precipitated NPs obtained from *P. stutzeri* at all concentrations and NPs synthesized by *T. harzianum* in a ratio of 1:50 were used. As for the effect of NP preparations on ChO of *P. roquefortii* activity, it was shown that preparations of *P. stutzeri* NPs both in conjunction with bacteria and separated from them had a stimulating effect. Thus, the co-incubation of non-precipitated nanoparticles taken at a ratio of 1:1000 and precipitated nanoparticles at a ratio of 1:100, 1:200 and 1:500 led to increasing in ChO activity by 1.14 times. The remaining studied concentrations led to inhibition of the activity of ChO by 1.75−3.50 times was observed or the activity of ChO remained at the control level. The use of NPs synthesized by *T. harzianum* led to a 2.3−3.5-fold decrease of ChO activity in all studied concentrations.

In case of using GOX of *P. adametzii*, the effect of NPs on the activity of the enzyme was insignificant, regardless what type of the microorganism had synthesized them. An increase in GOX activity of 5-6 % was observed with the addition of *P. stutzeri* and *T. harzianum* NPs in a ratio of 1:100; in other cases, NPs did not affect the activity of the enzyme.

*The research was conducted within the framework of the project B21UZB-018 (MRB-2021-554) financially supported by Belarusian Republican Foundation of Basic Investigations.*

# DEVELOPMENT OF GENETIC VECTORS BASED ON ARTEMISININ BIOSYNTHESIS RELATED GENES AND THEIR TRANSFORMATION INTO PLANTS USING AGROBACTERIUM

Rakhmanov B., Imamkhodjaeva A., Usmanov D., Ubaydullaeva Kh.,
Mirzakhmedov M., Shermatov Sh., Buriev Z.
*Center of Genomics and Bioinformatics, Academy of Sciences of the Republic of Uzbekistan, Uzbekistan*
e-mail: bakhtiyor.rakhmanov@gmail.com

CZU:615.322:582.998.1:575.16 https://doi.org/10.52757/imb22.05

Artemisinin (chemical formula $C_{15}H_{22}O_5$) is the sesquiterpene alkaloid, and it is produced in glandular trichomes that are present on leaves, floral buds, and flowers of the artemisia crops, but it is in very low amount leading to problem to fulfill medicinal needs.

According to global studies, demand for artemisinin is growing fast, especially to use it in medicine to cure the patients with malaria, various forms of cancer, and viral diseases COVID-19. Therefore, in recent years there have been reports in scientific publications that attempts have been made to develop more productive plants and microorganisms to obtain products with high yielding artemisinin using synthetic biology, genetic engineering and biotechnologies. Thus, using transcriptomics, metabolomics and genetic engineering data analysis and publications, in our study we developed genetic vector constructions based on the genes that participate in artemisinin biosynthesis.

To develop vector construction, we used the SnapGene software system, which is easy to use and rich in qualitative functionalities, as well as its visualisation helps to build vectors very effectively. Ready to transformation vectors into variety plants were confirmed for their accuracy by colony PCR, restriction enzymes and sequencing analysis. Explants of several plants such as leaves, cotyledons, and stems were subjected to our study to transform into new generated genetic vectors. Genetic transformation of vectors was realized with the help of Agrobacterium tumefaciens strains in aseptic conditions. Now after co-cultivation procedures steps of somatic embryogenesis such as callusogenesis, their differentiation, shoot and root formation under research in in vitro laboratory.

As the next steps of our research, we will obtain transgenic plantlets where integrated new genes can be expressing artemisinin or its related metabolites. The results of this study will be reported next after deep studies.

**FFECT OF ZINC-CONTAINING WASTEWATER ON *SPIRULINA PLATENSIS* BIOACCUMULATION CAPACITY AND BIOCHEMICAL COMPOSITION**

Zinicovscaia I.[1,2*], Cepoi L.[3], Rudi L.[3], Chiriac T.[3], Grozdov D.[1], Vergel K.[1]
[1]*Joint Institute for Nuclear Research, Russian Federation*
[2]*Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, Romania*
[3]*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: inga@jinr.ru

CZU:579.2:582.232 https://doi.org/10.52757/imb22.06

Cyanobacteria *Spirulina platensis* due to its high biosorption and bioaccumulation capacity toward metal ions can be considered as an excellent candidate for environment bioremediation. The effect of Zn and Zn-accompanying heavy metals in different combinations on the accumulation capacity of *S. platensis* biomass and its biochemical composition was investigated.

Four Zn-containing systems with a different combination of metal ions (Zn; Zn/Cu/Sr; Zn/Cu/Ni; Zn/Cu/Sr/Ba) and different metal concentrations were modelled. Studied systems were introduced in the cultivation medium on the fifth day of biomass grow and experiments were performed in three variants, which differed by metal ions concentrations. Metal uptake by biomass was traced using neutron activation analysis.

*S. platensis* showed high accumulation capacity for all metal ions present in the analyzed system. Because the metals were added at the beginning of the stationary growth phase, and the contact with the biomass was only 24 hours, even at the highest metal concentration in the systems accumulation of *S. platensis* biomass was reduced by no more than 11.2%. *S. platensis* biomass grown in a mono-metallic system expressed two biochemical indicators of stress: decrease of phycobiliproteins content and increase of malondialdehyde content. In biomass grown in the presence of Zn-containing multi-metallic systems three indicators of stress were expressed: decrease of proteins content, reduction of phycobiliproteins content and increase of malondialdehyde content. *S. platensis* biomass can be considered as an effective accumulator for the treatment of Zn-containing industrial effluents.

# INFLUENCE OF CULTIVATION CONDITIONS ON THE GROWTH AND FORMATION OF PSEUDOARTROBACTER SCLEROMAE CHOLESTEROL OXIDASE

Zhukouskaya L., Sudakova K., Semashko T.
*Institute of Microbiology of the National Academy of Sciences of Belarus, Belarus*
e-mail: mila_zhu@mail.ru

CZU:579.61:616.1 https://doi.org/10.52757/imb22.07

Cholesterol oxidase (ChO) (EC 1.1.3.6.) is a monomeric bifunctional flavin adenine dinucleotide (FAD)-dependent enzyme belonging to oxidoreductase family and catalyzing oxidation of 3β-hydroxysteroids and isomerization of intermediate cholest-5-en-3-one to cholest-4-en-3-one to yeld hydrogen peroxide. Cholesterol oxidase catalyzes the initial degradation of cholesterol and probably other natural sterols used as carbon sources for growth of various microorganisms.

ChO is engaged in clinical diagnostics to evaluate cholesterol level in blood, other biological fluids, foodstuffs, in fabrication of biosensors, precursors of steroid hormones, as insecticidal and antimicrobial agents.

According to literature data, the main ChO producers are strains referred to genera *Rhodococcus, Streptomyces, Brevibacterium.*

Taking into account a broad spectrum of enzyme application, the studies to seek new highly active sources of ChO are extremely relevant and vital.

Earlier we performed screening of bacterial strains synthesizing extracellular ChO using plate method based on growing bacteria on differential-diagnostic medium containing cholesterol and selection of the variants showing the maximum enzyme generation activity.

Aim of the study – investigation of the effect of cultural conditions on growth of *Pseudoarthrobacter scleromae* and ChO production.

As a result, the impact of initial pH of the nutrient medium (5,0-10,0), temperature (26-30°C), time of culture (48-96 h) was assessed.

Analysis of growth and ChO biosynthetic capacity by *P. scleromae* showed that in the course of fermentation active acidity of the nutrient medium tended to rise by 0,36-2,71 from the initial pH value 5,0-9,0 and feel by 0,55 from the starting pH 10,0, staying within the range 7,71-9,45. Protein concentration upon the end of fermentation reached 8,21-14,05 mg/ml. It should be noted that this parameter was sliding up with the increase of initial pH of the medium. As to biomass concentration, its level varied by the final day of the culture from 27,86 to 30,97 mg/ml. The top ChO generation capacity was recorded at initial pH of the medium 8,0 (0,088 U/ml). Initial pH values 5,0 and 6,0 decreased ChO productivity 2-fold (0,044 U/ml). Further pH fluctuations resulted in more drastic losses of enzyme biosynthesis.

Examination of the effect of temperature on enzyme production revealed that peak amount of ChO was generated at 28°C.

The study on correlation of enzyme productivity with time of the culture demonstrated that optimal period for ChO biosynthesis equaled 72 h. More prolonged culture lasting up to 96 h failed to augment enzyme yield.

Thus, it may be concluded that the optimal parameters for growth of *P. scleromae* and secretion of extracellular ChO are 72 h culture at temperature 28°C and initial pH of the nutrient medium 8,0.

**BIOACTIVE GLASS COATINGS SYNTHESIZED BY "MAPLE" FOR ENHANCED PERFORMANCE OF MEDICAL IMPLANTS**

Ungureanu (Negut) I.[1], Ristoscu C.[1], Tozar T.[1,2], Grumezescu V.[1], Dinu M.[3], Parau A.C.[3], Popa M.[4], Stan M.S.[5]

*[1] National Institute for Lasers, Plasma and Radiation Physics, Romania,*
*[2]Extreme Light Infrastructure-Nuclear Physics, Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, Romania;*
*[3]National Institute of Research and Development for Optoelectronics, Romania;*
*[4]Faculty of Biology, Microbiology Immunology Department, University of Bucharest, Romania;*
*[5]Faculty of Biology, Department of Biochemistry and Molecular Biology, University of Bucharest, Romania*
e-mail: negut.irina@inflpr.ro

CZU:579.61:616-089.843 https://doi.org/10.52757/imb22.08

We deposited thin films from bioglass/antibiotic by the matrix-assisted pulsed laser evaporation (MAPLE) technique onto metallic substrates which mimic the surfaces of medical implants. The deposition was made in a two-step procedure: i) a thin layer of the polymeric material was deposited by MAPLE onto Ti substrates, ii) a second layer consisting of bioglass+antibiotic was applied by MAPLE onto the prior deposited polymeric film.

The surface morphology of samples was examined by SEM and the surface topography was assessed by AFM. The wettability of the obtained thin films was studied by means of the sessile drop method, whereas the chemical functions integrity of thin films was studied FT-IR. To simulate the insertion of implants into the physiological media of the human body and the phenomena happening at the tissue-implant interface, samples were immersed in SBF and investigated by FT-IR, after different times. The SBF solutions containing the released products from thin films were analysed by UV-Vis. The electrochemical behaviour of the investigated samples was analysed by potentiodynamic polarization and electrochemical impedance spectroscopy. The antimicrobial action of the antibiotic-containing thin films was evaluated on *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* standard strains. The biocompatibility of obtained thin films was assessed on mouse osteoblast-like cells.

The laser-deposited coatings were biocompatible and resistant to microbial colonization and biofilm formation and can be taken into consideration as novel and viable candidates for implantable surfaces.

*Acknowledgment: This work was supported by the Romanian Ministry of Education and Research, under Romanian National Nucleu Program LAPLAS VI—contract no 16N/2019. M.D., A.C.P. acknowledges the support of the Romanian National Core Program 18N/2019. I.N. acknowledges the support by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN- PN-III-P1-1.1-PD-2021-0598 and PN-III-P2-2.1-PED-2021-3178, within PNCDI III. T.T. acknowledges the support by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P1-1.1-PD-2019-1117, within PNCDI III.*

# THE CORRELATION BETWEEN THE IMMUNE INDICATORS IN PATIENTS WITH PULMONARY DRUG-SUSCEPTIBLE AND MULTIDRUG-RESISTANT TUBERCULOSIS

Lesnic E.[1], Ghinda S.[2], Privalova E.[2] Niguleanu A.[1]
[1] *State Medicine and Pharmacy University Nicolae Testemitanu, Republic of Moldova*
[2] *Immunological and allergological laboratory of Pneumophtysiology Institute "Chiril Draganiuc" Republic of Moldova*
e-mail: evelina.lesnic@usmf.md

CZU:616.24 -002.5:612.017:[616-08-078] https://doi.org/10.52757/imb22.09

Evolution of tuberculosis (TB) and outcome reflect the mycobacteria (MBT) virulence and the organism's capacity to involve the main immune response, as the cell-mediated response (CMR) involving the main effectors - CD3+ and CD4+ lymphocytes. The innate immunity, with the role to balance the protective and pathogenic immune factors, leads to the lung destruction and chronic inflammation. Its effectors are macrophages, dendritic cells, neutrophils and natural killer cells. The humoral immunity (HI) has a secondary role in the response against to MBT synthetizing the antibodies by CD19+ lymphocytes. **The aim** was to determine the correlation between cell-mediated immunity, humoral immunity, and innate resistance indices in patients with drug-susceptible TB and multidrug-resistant TB (MDR-TB). **Material and methods**: a prospective, case-control study included 129 patients diagnosed with pulmonary TB, from which 57 new cases of drug-susceptible pulmonary TB consisted the control group (CG) and 72 cases of MDR-TB – the study group (SG) which was distributed in the 1st SG with 41 cases with primary MDR-TB and the 2nd SG with 31 acquired MDR-TB. The immune assays included the reaction of blast transformation of lymphocytes (RLBT) by phytohemagglutinin (PHA) and tuberculin (PPD), immunophenotyping of CD3+ and CD19+ cells, phagocytic number (PN). **Results:** A highly strong and postive correlation was identified between levels of RLBT by PHA and CD3+ in all groups; a low and postive correlation between levels of LBTR by PPD and CD3+ in all groups; moderate negative correlation between levels of RLBT to PHA and CD19+ in all groups, low negative correlation between levels of LBTR by PPD and CD19+ in all groups; and moderate negative correlations between PN and RLBT by PHA in all groups, as well as between PN and RLBT by PPD in all groups, low negative correlation between PN and CD3+ in all groups, moderate negative correlation between PN and levels of CD19+ in all groups (Table 1).

**Table 1. Correlation between immunological indices in drug-susceptible and MDR-TB**

| Correlated indices | CG | 1st SG | 2nd SG |
|---|---|---|---|
| RLBT by PHA and CD3+ | r=0,67; p=0,001 [2] | r=0,71; p=0,001 [3] | r=0,78; p=0,001 [3] |
| RLBT by PPD and CD3+ | r=0,38; p=0,01 [2] | r=0,42; p=0,01 [2] | r=0,48; p=0,01 [2] |
| RLBT by PHA and CD19+ | r=0,65; p=0,001 [2] | r=-0,71; p=0,001 [3] | r=-0,71; p=0,001 [3] |
| RLBT by PPD and CD19+ | r=-0,29; p=0,01 [1] | r=-0,32; p=0,01 [2] | r=-0,37; p=0,01 [2] |
| PN and RLBT by PHA | r=-0,59; p=0,001 [2] | r=-0,62; p=0,001[2] | r =0,67; p=0,01 [2] |
| PN and RLBT by PPD | r=-0,39; p=0,01 [2] | r=-0,44; p=0,01 [2] | r=-0,47; p=0,01 [2] |
| PN and CD3+ | r=-0,29; p=0,05 [2] | r=-0,32; p=0,01[2] | r=-0,44; p=0,01[2] |
| PN and CD19+ | r=-0,59; p=0,05 [2] | r=0,44; ; p=0,01[2] | r=-0,67; p=0,01[2] |

**Conclusions:** The indices of cell-mediated immunity positively correlated between them (RLBT by PHA, PPD and CD3+); positively RLBT by PHA and negative RLBT by PPD and CD3+; negatively correlated CD3+ and CD19+ with PN, more evident in MDR-TB, especially in acquired MDR-TB.

GREEN

# Biotechnology

Agricultural and Environmental Biotehnology

**ANTIOXIDANT CAPACITY OF THE EXTTHE *ACTINOBACTERIA* MICROBIAL COMMUNITY STRUCTURE IN A TYPICAL CHERNOZEM SOIL**

Artiomov L.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: lara_09@rambler.ru

CZU:579.64:631.461 https://doi.org/10.52757/imb22.10

*Actinobacteria,* the biotechnologically valuable bacteria, are the dominant class of the *Bacteria* domain in most soils. Approximately 45% of all discovered bioactive microbial metabolites are produced by *Actinobacteria*.

The aim of the research was to study the community structure of *Actinobacteria* class from a typical chernozem. The research was carried out in the long-term field experiment of the "*Biotron*" Experimental Station of the Academy of Sciences of Moldova in two crop rotations (with and without alfalfa). Characterization of the compositional diversity of the soil microbiome was achieved by the 16S rRNA amplicon sequencing (Scientific Center "Genomic Technologies, Proteomics and Cell Biology" of FSBSI ARRIAM, St. Petersburg, Russia).

The results of the investigations demonstrated that *Actinobacteria* had the highest relative abundance (8.2%) compared to 3 other classes (*Thermoleophilia*, *Rubrobacteria*, *Acidimicrobiia*) of the phylum *Actinobacteriota*, identified in the soil of the the long-term field experiment of the "*Biotron*" Experimental Station (Chişinău ). *Actinobacteria* had the highest relative abundance in the *Mineral fertilization* and *Control* variants of both crop rotations, and the lowest abundance was in the *Organic fertilization* (postaction) variant. The cla*ss* was represented by 10 orders of bacteria. The orders *Propionibacteriales (2.5%), Micrococcales* (2.3%) and *Frankiales* – (1.2%) had the highest relative abundance. The order *Propionibacteriales* was more abundant in the variants with mineral fertilization of the both crop rotations, and in the soil of the forest shelterbelt, the order *Micrococcales* - in the variants *Control* and *Forest shelterbelt*, the order *Frankiale*s - in the variants *Control* of the both crop rotations, and *Mineral fertilization* of the crop rotation without alfalfa. The rarer orders with abundance ≤ 0.1 were *Corynebacteriales, Kineosporiales*, *Streptosporangiales*. The maximum abundance of these rare orders was observed in the following variants: *Corynebacteria*les - in the *Forest shelterbelt*, *Kineosporiales* - in *Control* of the both crop rotations, and *Streptosporangiales* - in *Mineral fertilization* of the crop rotation with alfalfa. The order *Streptosporangiales* had the lowest abundance in the soil of the *Forest shelterbelt* (<0.01%).

The orders included 20 families, and 34 genera. Most genera (7) were identified in the *Pseudonocardiaceae* family. The most abundant genera were: *Microlunatus* – 1.5% (the *Propionibacteriaceae* family), *Blastococcus* – 0.9% (*Geodermatophilaceae*), *Agromyces* – 0.5% (*Microbacteriaceae*), *Pseudonocardia* – 0.4%, (*Pseudonocardiaceae*), *Streptomyces* – 0.4% (*Streptomycetaceae*). The genus *Microlunatus* was present in all variants of the experiment with the abundance > 1%, it included 25 species of aerobic, chemo-organotrophic bacteria, some species can oxidize nitrates in anaerobic conditions and accumulate phosphates. The genus *Blastococcus* (12 species) had the lowest abundance in the uncultivated land of the *Forest shelterbelt*, and the highest abundance was determined in the *Control* and *Mineral fertilization* variants of the both crop rotations. The genus *Agromyces* (45 species), considered as an indicator of healthy soils, reached the maximum abundance in the uncultivated soil of the *Forest shelterbelt* and the unfertilized soil of the *Control* variants of the both crop rotations.

The metagenomic research of the typical chernozem demonstrated the presence of a great diversity of *Actinobacteria* with biotechnological potential both in the soils of the agricultural plots and of the forest shelterbelt. Finding correlations between the applied agricultural practices and the *Actinobacteria* diversity requires a further detailed study.

*This research was carried out within the State Program Project 20.80009.5107 (2020-2023), financed by ANCD.*

# ENDOLYTIC MICROORGANISMS FROM VOLCANIC TUFF

Batrinescu-Moteau C.[1,2], Ruginescu R.[1,2], Neagu S.[1], Cojoc R.[1], Lucaci A. I.[1], Vlad A.[1], Purcarea C.[1], Enache M.[1,2]

[1] *Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independentei, 060031 Bucharest, Romania*
[2] *SC Medacril SRL, Carpati street no 8, Medias, Sibiu, Romania*

CZU:561.2:552.313.8 https://doi.org/10.52757/imb22.11

The abundance and diversity of microorganisms in the biosphere reflects their ability to accumulate energy from various organic and inorganic substrates as well as the ability to grow in a wide range of natural conditions. Most often, the complexity of microbial communities is dependent on the conditions offered by the habitat for its colonization and the availability for energy and carbon sources. In most cases, habitats offer extreme climatic or environmental conditions, thus limiting the number of species that may exist in such an ecological niche. An example of such natural selection can be found in endolytic habitats which are colonized by microorganisms involved in their biodegradation or biodeterioration. This paper is a holistic approach to such microorganisms and the types of substrates they can colonize.

**CONNECTIONS BETWEEN MICROBIOTA, THE ENVIRONMENT AND THE REPRODUCTIVE HEALTH**

Balan I., Balacci S., Rosca N., Buzan V., Cazacov I., Moroz M., Osipciuc G., Cretu R., Bacu Gh.
*Institute of Physiology and Sanocreatology, Republic of Moldova*
e-mail: balanion@rambler.ru

CZU:579.63+504+612.6 https://doi.org/10.52757/imb22.12

The evolution of macroorganisms continuously takes place in favorable environments and in close correlation with microorganisms. The body's biological processes take place in the association with the microbial communities that have evolved within the hosts, commonly referred to as the body's microbiota. The microbiota is a valuable factor for estimating physiological states in reproduction, which is essential for the survival. Balanced interactions within and between cells of the host organism and non-host resident microbial community in the external environment are essential for reproductive health. The reproductive microbiota represents the microbial communities that populate the male reproductive tract and directly influence reproductive success. The microbiota associated with the reproductive tract is influenced by both the endogenous physiological processes and variations in the exogenous environmental factors. The main objective of this review Is to highlight the interactions between the reproductive biology of animals, their microbiota and the environmental factors. Establishing the link between microbiota, the reproduction, and the environment helps to fill important gaps in the reproductive microbiota research.

In males, the semen is a complex biological fluid that contains nutrients for sperm, as well as the factors that influence sperm motility and other morpho-functional properties. The content of semen serves as an adaptive and favorable environment for microorganisms. Some microbes are associated with a reduced sperm quality, while others are associated with a higher quality sperm, suggesting that microorganisms may serve as indicators of sperm quality. The host metabolism has effects on the semen and its microbiota with further effects for the reproduction. the microbial community of the semen remains at least for a short time in the female reproductive tract after copulation, with slight modification of the vaginal microbiota, and may influence fertilization and perhaps even implantation.

The effects of beneficial microbiota vary between the host organisms and are undeniably a vital aspect of conservation breeding. The pollution and contamination of natural environments can influence the microbiota and affect the reproductive success. Some man-made compounds in natural ecosystems can alter both environmental and host-associated microbial communities, and pose significant risks for the reproductive health. At the same time, the study of the reproduction in the natural ecosystems opens possibilities for microbiota conservation and global microbial diversity preservation. The study and conservation of the reproductive microbiota is particularly relevant to the biodiversity-managed breeding programs.

Most research to date has been focused on the gut microbial communities, and only few studies examined changes in the reproductive microbiota. Further study of the reproductive microbiota may elucidate potential mechanisms of dysbiosis and, in turn, provide opportunities for integrating the microbial ecology into the reproductive practices.

A part of the microbiota present in the reproductive tract can contribute to the facilitation of reproduction. In many cases it is hard to know whether the changes in the microbiota cause the changes in the reproductive state of the host, or whether they are a by-product of physiological changes. In addition, the host-associated microbiota is influenced by exogenous factors of the environment variation, which require additional research into optimization of the breeding and conservation efforts.

**CHARACTERIZATION OF THE MANNOPROTEIN EXTRACTS OBTAINED FROM WINE INDUSTRY WASTE**

Besliu A., Chiselita N., Chiselita O., Efremova N., Tofan E., Sprincean A.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: besliu.imb@gmail.com

CZU:579.222:579.66:663.26 https://doi.org/10.52757/imb22.13

Currently, special attention within the scientific research is paid to the utilization of the waste from industrial production. In the Republic of Moldova of a particular interest is the utilization of the great quantities of the yeast sediments left after the wine production. The relevance of this direction is conditioned by the need to solve the problem of the discarded waste that damages the environment [2], and by the possibility of obtaining yeast extracts of a high biotechnological value. The mannoproteins are an important component in the structure of the yeast cell wall, and, their molecular and structural properties make them attractive for application in the agriculture, especially in the animal husbandry, food, cosmetic and wine industries. Thus, the aim of the study was to characterize the mannoprotein extracts obtained from the waste products of the wine industry.

The yeast sediments from production of the dry white wine *Rkatsiteli* (SAR), as well as the red *Merlot* (SRM) and *Cabernet* (SRC) wines by the Cricova winery, were used as the research material. Autolysis of the yeast biomass was performed by using 3% acetic acid at the temperature of +55°C for 8 hours, sodium phosphate buffer at the temperature of +45°C for 8 hours, and homogenization for 10 minutes with subsequent storage at the temperature of +45°C for 8 hours. Alkaline mannoprotein extracts were obtained according to the method [1].

For the characterization of the obtained mannoprotein extracts, the biochemical composition and the activity of the antioxidant enzymes catalase and superoxide dismutase were determined. It was established that the protein content of the mannoprotein extracts prevailed over the carbohydrate content, regardless of the autolysis method used. Thus, carbohydrates varied within the limits of 11.1±0.2-47.9±0.2% (d.w.), and the protein content oscillated between 20.9±0.27 and 65.9±3.9% (d.w.), minimum values being established in the extracts obtained from the SAR biomass. Maximum values of the protein and carbohydrate contents were obtained in the experimental variants autolyzed with sodium phosphate buffer solution at the temperature of +45˚C, for 8 hours. The study of the enzymatic activity showed that the activity of catalase-type enzymes varied within the limits of 402.3±1.89-842.6±5.2 mmol/min./mg protein and the SOD activity was within the limits of 47.3±0.11-120.9±0.04 U/mg protein. The mannoprotein content, related to the absolutely dry biomass, varied within the limits of 17.9-43.2% with the maximum values established in the extracts obtained from the SRM biomass with the use of sodium phosphate buffer solution for the induction of autolysis.

The obtained results indicate that the use of biomass from the SRM wine sediments and the autolysis by sodium phosphate buffer solution at +45°C for 8 hours permitted to obtain mannoprotein extracts of a higher biochemical composition and enzymatic activity as compared to the other studied experimental variants.

**References:**

1. Beșliu, A., Chiselița, N., Chiselița, O., Efremova, N., Tofan, E.; Sprincean, A., Daniliș, M. Biochemical composition and antioxidant activity of the mannoprotein preparation obtained yeast biomass from wine industry waste. In: *Notulae Scientia Biologicae*. 2022, 14(2), 11229.
2. Devesa-Rey, R., Vecino, X., Varela, J., Barral, M., Cruz, J., Moldes, A. Valorization of winery waste vs. the costs of not recycling. In: Waste Manag. 2011, 31, (11), 2327-35.

*The research was carried out within the project 20.80009.5107.16 "New biologically active microbial preparations for increasing the reproductive and productive potential of animals of zootechnical interest", funded by NARD.*

# THE VIABILITY OF *BACILLUS*, *PSEUDOMONAS* AND LACTIC ACID BACTERIA STRAINS AFTER 15 YEARS OF STORAGE

Bogdan-Golubi N., Slanina V.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: nina.bogdan@imb.md

CZU:576.8+579.22+579.26 https://doi.org/10.52757/imb22.14

The National Collection of Nonpathogenic Microorganisms (NCNM) contains bacterial species like *Rhizobium* sp., *Pseudomonas* sp., *Bacillus* sp., which are known for their antimicrobial activity, plant stimulation effects, and exometabolites that can be used for plant protection. Some can be used for the insect and plant disease controls.

The Collection also contains the lactic acid bacteria isolated from naturally fermented homemade dairy foods, that can be used for obtaining sour cream, fresh cheese, yoghurt, soy milk, brined cheese. These bacteria permit to create a better taste, flavor and texture of the fermented foods, and to ensure manufacturing dairy products enriched with beneficial microorganisms, with an extended shelf-life and enhanced food safety of food products (due to the production of the lactic acid as an antimicrobial substance).

Cell viability during storage is of a great importance for the cultures used in the food and/or agriculture industries. Freeze-drying (lyophilization) provides a higher cell viability and is used for the long-term preservation. Depending on the resistance to the freeze-drying process there are three groups or bacteria: the resistant, the moderately resistant and the sensitive ones. According to this classification, bacteria from the *Pseudomonas* and *Bacillus* genus have been either resistant or moderately resistant to the lyophilization process.

For the NCNM just like for any other similar collection conservation and long-term storage of valuable microbial strains (fungi, yeasts, actinomycetes, bacteria, cyanobacteria, microalgae) is of a special importance.

The aim of the research was to check the viability and stability of the pure strains of *Bacillus* sp., *Pseudomonas* sp. and lactic acid bacteria strains a 15-year storage in the NCNM. Lactic acid bacteria included *Lactococcus* sp. and *Streptococcus thermophilus*.

The number of viable cells was determined as the colony forming units per ml (CFU/ml), and the survival rate was calculated as CFU/ml after freeze-drying divided by CFU/ml before freeze-drying.

The *Bacillus* sp. and *Pseudomonas* sp. strains were found to be viable and their titer ranged from 6,8 to 7,6 $\log_{10}$UFCml$^{-1}$ and from 7,9 to 8,1 $\log_{10}$UFCml$^{-1}$ respectively. It is known that the *Pseudomonas* and *Bacillus* bacteria can be stored for over 30 years in freeze-dried form with no changes in the high level cell viability at 6-7 $\log_{10}$UFCml$^{-1}$. Lactic acid bacteria strains after 15 years of storage in freeze-dried form had a survival rate of 80% with the titer ranged from 6,2 to 8,3 $\log_{10}$UFCml$^{-1}$. According to other studies the minimal viability of different species of *Streptococcus, Staphylococcus, Brevibacterium, Pseudomonas, Corynebacterium, Lactobacillus, Salmonella, Bacillus* after freeze-drying could reach 70%. Thus, the number of viable cells remaining in the tested ampoules was sufficient to maintain the culture.

Microscopic examination confirmed the purity of the cultures. *Bacillus* sp. Was represented by rod-shaped Gram-positive cells, and *Pseudomonas* sp - by Gram-negative. Lactic acid bacteria were present as cocci in short or long chains. All their strains were able to cause active milk coagulation, producing dense consistence, without gas eruption, and, therefore, respected the technological requirements for the lactic acid bacteria species.

The obtained results confirmed the effectiveness of freeze-drying for the tested strains

*The research was funded by NARD within project 20.80009.7007.09*

**ANTOCYANIC EXTRACTS FROM YEAST WINE WASTE**

Chiselita O.[1], Chiselita N.[1], Tofan E.[1], Besliu A.[1], Efremova N.[1], Danilis M.[1], Rotaru A.[2]
[1]*Institute of Microbiology and Biotechnology, Republic of Moldova*
[2]*State Agrarian University of Moldova*
e-mail**:** oleg.chiselita@imb.md

CZU:663.26:547.819 https://doi.org/10.52757/imb22.15

Phenolic compounds, especially phenolic acids, tannins and anthocyanins are among the important biologically active components of wines. Of all the phenolic compounds, anthocyanins are of a particular interest because they have many beneficial effects on human and animal health. *In vitro* and *in vivo* studies have revealed the biological potential of these compounds and demonstrated their ability to reduce oxidative stress, to act as antimicrobial substances and to counteract the appearance and progression of many non-transmissible diseases, such as neurodegenerative, cardiovascular, metabolic ones and cancer. In combination with vitamin A and other carotenoids they protect visual function. Anthocyanins and their derivatives have no toxic effect on living organisms, even after ingestion in very high doses.

Since the biologically active substances, including anthocyanins, found out in the fermentation medium, are largely absorbed on the surface of the yeast cells, yeast biomass remaining from wine production, can serve as an important source of these substances.

The purposes of this research were to obtain anthocyanin extracts from the yeast biomass remaining from the production of the autochthonous wines, to characterize them biochemically, and to assess their antioxidant potential. The research was focused on the sediment yeast biomass from the production of white dry wine *Rkatsiteli*, red dry wines *Merlot* and *Cabernet*, offered by the «Cricova» winery.

The extracts were obtained by different methods of destruction of the yeast cell wall, which included the use of the acetic acid and the sodium phosphate buffer solutions, homogenization, different temperatures and biomass-solution ratios. The extracts were characterized by their dry weight, by the content of the anthocyanins, proteins, and carbohydrates, as well as by the activity of the antioxidant enzymes catalase and superoxide dismutase.

Depending on the yeast biomass type and the cell wall destruction method the obtained extracts had the dry weight of 2.2 - 13.3 mg/ml, and contained 3.9±0.3 - 20.7±0.4 mg/g of cyanidin anthocyanins, 3.2±0.2 - 9.7±0.4% (d.w.) of proteins, 2.2±0.02 - 31.4±0.3% (d.w.) of carbohydrates, and possessed the antioxidant type catalase activity of 315±2.6 - 524±1.5 mmol/min/mg protein and the superoxide dismutase of 173±5.2 - 457±0.6 U/mg protein.

The valuable biochemical composition and high activity of the antioxidant enzymes such as catalase type and superoxide dismutase of the extracts revealed the perspectivity of using the yeast biomass from wine production as a source of anthocyanin preparations for various fields.

The results of the research permitted to elaborate a procedure of obtaining the anthocyanin preparations from yeast biomass after red wine fermentation, which is currently being patented.

*Acknowledgements: The results were obtained within Project 20.80009.5107.16. "New biologically active microbial preparations for increasing the reproductive and productive potential of animals of zootechnical interest", funded by the National Agency for Research and Development.*

# CHROMIUM BIOACCUMULATION POTENTIAL OF EDAPHIC CYANOBACTERIUM *NOSTOC LINCKIA* GROWN ON MULTIMETALLIC SYSTEMS

Codreanu L.
*Institute of Microbiology and Biotechnology, Chisinau, Republic of Moldova*
e-mail: co_liv@mail.ru

CZU:579.69+579.222+57.04 https://doi.org/10.52757/imb22.16

Over the past few decades, chromium contamination of both terrestrial and aquatic ecosystems has increased as a result of various anthropogenic activities. In this regard, various useful and practical remediation technologies have been emerging to control chromium content in water, soil and other resources. Chromium remediation through microorganisms may be the best technology currently available for cleaning up Cr contaminated sites. These technologies using biological agents are cheaper, safer and eco-friendly than chemical treatment methods. Cr exists in several oxidation states, but the most stable and common forms are Cr(0), Cr(III) and Cr(VI) species. Chromium toxicity depends on its valence state. *Hexavalent chromium is a* highly mobile and toxic contaminant. Cr(III) being less mobile is much less toxic *than Cr*(VI).

The purpose of this study was to assess the potential of cyanobacterium *Nostoc linckia* to accumulate hexavalent chromium during three successive cultivation cycles on multimetallic systems. Cyanobacterium *Nostoc linckia (Roth) Born et Flah* CNM-CB-03 was grown in a mineral medium and metal ions in different combinations. Cultivation was carried out in Erlenmayer flasks of 1000 mL with a working volume of 700 mL. The following parameters were used: pH of the medium 6.8-7.2, temperature 25-27ºC, light intensity of 37-55 µmol photons $m^{-2}$ $s^{-1}$, continuous illumination, slow periodic shaking. The amount of inoculum was 0.4 g/L. Each cultivation cycle lasted 12 days. Multimetallic systems Cr/Fe, Cr/Fe/Ni, Cr/Fe/Ni/Zn and Cr/Fe/Ni/Zn/Cu were added to culture medium on the exponential phase of nostoc growth.

In this study, the bioaccumulation of Cr(VI) from multimetallic systems was performed - a situation that is closer to real conditions, since in most cases the contamination of certain areas occurs due to the presence of several pollutants. Among the four studied systems, in Cr/Fe, Cr/Fe/Ni, and Cr/Fe/Ni/Zn/Cu, the rate of chromium uptake by nostoc was very similar and varied with the cultivation cycle. Thus, in these three systems, chromium uptake in the first cultivation cycle was 35.8-40.2% of the initial level of metal; in the second cycle - 27.2-32.7%, and in the third cycle - 19.7-27.1%. In Cr/Fe/Ni/Zn system, the bioaccumulation capacity of nostoc biomass was significantly higher and amounted to 63.1%, 56.0% and 34.8% of Cr(VI), which corresponds to three cultivation cycles. Moreover, Fe, Ni, Zn and Cu were also taken up during the cultivation of nostoc biomass from one cycle to another. For instance, iron uptake in the first cycle was 59-78% and it was accumulated almost completely in the next two cycles. Nickel uptake in the first cycle was 43.2-62.6%, in the next two - 49.7-83.1%. The percentage of zinc recovery by nostoc biomass was at the same level in all three cycles and amounted to 37.1-39.8% of its initial content in the Cr/Fe/Ni/Zn system. In the Cr/Fe/Zn/Ni/Cu system, zinc uptake increased from 25.8% in the first cycle to 54.5% in the third one. Copper uptake was 46.5-57.8%, and its maximum amount was accumulated in the second cycle of nostoc cultivation.

Thus, the culture of *Nostoc linckia* demonstrated resistance to multimetallic systems and a high potential for bioaccumulation of Cr(VI) and other metals present. The capacity of cyanobacterium *Nostoc linckia* to bioaccumulate Cr(VI) from the contaminated medium remained high over three generations, while the uptake of Fe, Ni, Cu and Zn in the biomass increased from generation to generation.

In conclusion, edaphic cyanobacterium *Nostoc linkia* is a good accumulator of chromium, but also of other metals in multimetallic systems. Due to its biological nature, *Nostoc linckia* is a suitable matrix for remediation processes that offers a vast competition ground for metal cations. Therefore, the use of *microorganisms for heavy metal removal* is a sustainable remediation approach that must be adopted in order to balance the environment and nature.

# SOME ASPECTS REGARDING THE MICROORGANISMS INVOLVED IN BIODEGRADABLE WASTE COMPOSTING

Dudnicenco T.
*Moldova State University, Department of Biology and Ecology, Republic of Moldova*
e-mail: dudnicenco@yahoo.com

CZU:579.695:628.381.1 https://doi.org/10.52757/imb22.17

Composting is one of the ecological methods of processing biodegradable waste via its transforming into a non-polluting product called compost, with high nutritional value for plants, which can be used for improving the physical and chemical properties of soils. Composting can be used in parallel or instead of the incineration and controlled storage techniques. The advantages of this method include: reduction of the waste volume, turning the waste into a product which is useful for the Republic of Moldova – compost for agriculture, horticulture and gardening.

Microorganisms (bacteria and fungi) have the leading role in the process of composting biodegradable waste. The involved bacteria and fungi can be classified into mesophiles and thermophiles. The mesophilic microorganisms (those that grow best at temperatures between 25-45°C) are dominant in the composting mass in the initial phase of the process when temperatures are relatively low. They use available oxygen to convert carbon from compostable materials into energy, thus producing $CO_2$, $H_2O$ and heat as a result of metabolic processes. When the temperature approaches 45°C, the mesophilic microorganisms die or become inactive, waiting for the preferable conditions. The thermophilic microorganisms are active at temperatures between 45-70°C, when they rapidly multiply replacing the mesophiles in most sections of the compost pile. They usually appear after 5-10 days of composting and generate a much higher heat than the mesophylls. The temperatures reached with their help are high enough to inactivate most pathogens and weed seeds. The thermophilic bacteria continue to break down materials as long as there are nutrients and energy sources. When these sources diminish, the thermophiles die and the temperature in the piles begins to decrease. At this point, the mesophilic bacteria resume their activity in the decomposition process until all available sources of nutrients and energy are exhausted.

Bacteria are the first to colonize the compost pile and they break down primary proteins and carbohydrates faster than the other types of organisms. Among them there are *Bacillus mesentericus, Bacillus vulgatus, Bactehum vulgarae, Bacterium fluorescens, Micrococcus sulfureus, Micrococcus luteus, Streptococcus yogenes* etc., nitrifying bacteria, myxobacteria and pathogenic bacteria. The bacterium *Pseudomonas aeruginosa* is the most common microorganism in the composting process.

Fungi have an important role in composting too, but only when the pile starts to dry, because they can survive the environments with low humidity more easily than bacteria. Some types of fungi may require a smaller amount of nitrogen compared to bacteria, being thus able to break down cellulosic materials that bacteria cannot break down. Among the important fungal species, a lot of attention was given to the ones belonging to the genera *Aspergillus*. Among other important species are: *Mucor, Chaetocladium, Thamnidium, Microacus, Absidia, Helicostylum, Chaetomium, Circinella, Motierella* etc.

The factors that influence the composting process must be kept under control, namely: the composition of the residues, the moisture content of the waste, the oxygen concentration, the temperature. Among the auxiliary factors that influence the composting process, we can mention the homogeneity of the mixture; waste granulation; the way of placing the ground waste in piles or in fermentation containers.

The composting can be effectively used for neutralization of household waste, since it is much cheaper than storage in controlled ecological deposits or incineration. More than that, the use of composts in the agriculture can cause a 15% yield increase.

# Characterization of some raw materials from several sources for obtaining biodegradable packging

Enache M. [1,2], Batrinescu-Moteau C. [1,2], Ruginescu R. [1,2], Neagu S.[1], Cojoc R. [1], Lucaci A. I. [1], Vlad A.[1], Purcarea C. [1]

[1] *Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independentei, 060031 Bucharest, Romania*
[2] *SC Medacril SRL, Carpati street no 8, Medias, Sibiu, Romania*

CZU:547.458.61+621.798-35 https://doi.org/10.52757/imb22.18

Plastic materials in contact with food products must be manufactured in accordance with good manufacturing practices (GMP) and not transfer their components into food products in quantities that could endanger human health, change the composition of food products in a way unacceptable, or to damage the taste and smell of food products. An in-depth analysis of the results will lead to the definition of industrial specifications for packaging materials, in accordance with the needs, limitations and processing demands of existing plastic materials and with the performances required for the specified products to be introduced to the market. The current state of knowledge in the field of biodegradable food packaging will be investigated. The technical requirements for obtaining bioactive food packaging in comparison with conventional plastic food packaging will be defined.

## *STREPTOMYCES FRADIAE* CNMN-Ac-11 AFTER STORAGE BY SUBCULTURING AND CULTIVATION ON COMPLEX MEDIA

Garbuzneac A., Birsa M., Burtseva S.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: 11_lav_11@mail.ru

CZU:579.873.7:577.115 https://doi.org/10.52757/imb22.19

Actinobacteria of the genus *Streptomyces* are known as producers of antibiotics, enzymes, hormones, vitamins, antipsychotics, antitumor agents, vaccines for humans and animals, growth stimulants and other substances that are used in medicine, veterinary medicine, agriculture and many other fields.

In recent years, studies have been focused on increasing the production of bioactive metabolites of promising streptomycete strains via optimization of the cultivation conditions.

The aim of the study was to determine the composition of lipids in the biomass of the *Streptomyces fradiae* CNMN-Ac-11 strain after cultivation on complex media after long-term storage by subculturing.

It was found that when this strain was cultivated on the M-I medium, the biomass yield was 6.09 g/l. On the SP-I medium with the addition of 3.0 g of $K_2HPO_4$ (SP-III), the biomass yield increased to 9.61 g/l. The percentage of total lipids in the biomass of the *Streptomyces fradiae* CNMN-Ac-11 strain on the SP-I medium was 19.52%, and on the M-I and SP-III media – 8.76% and 12.76% respectively.

Analysis of the studies in the past showed that the long-term storage could affect the formation of biomass and total lipids during the cultivation of the *Streptomyces fradiae* CNMN-Ac-11 strain on the M-I complex medium. Thus, according to the results in 2015, the biomass yield was 14.15 g/l, which is significantly higher than 6.09 g/l obtained in 2019. The proportion of total lipids in the biomass during the cultivation of the *Streptomyces fradiae* CNMN-Ac-11 strain was 12.11% in 2015, and 8.76% in 2019.

After the long-term storage by subculturing, the cultivation of the *Streptomyces massaporeus* CNMN-Ac-06 strain on the M-I medium increased the biomass yield to 7.18 g/l, and the *Streptomyces fradiae* CNMN-Ac-11 strain – to 6.09 g/l. Regarding the accumulation of total lipids, it was noted that the best result was shown by the *Streptomyces fradiae* CNMN-Ac-11 strain (8.76%), in contrast to the *Streptomyces massaporeus* CNMN-Ac-06 strain (4.96%).

It was also found that after the storage by periodic transfers and cultivation on the M-I complex medium, there was a decrease in phospholipids (4.31%) and triglycerides (13.55%) that occurred simultaneously with an increase in sterols (12.97%), which was probably due to the changes in the medium composition, where corn flour was the main source of carbon, as well as due to the high heterogeneity and individual characteristics of streptomycetes.

It was experimentally shown that to increase the biomass yield the *Streptomyces fradiae* CNMN-Ac-11 strain better be cultivated on the complex media SP-I and SP-III, which also contribute to increases in the lipid content of the biomass, and, most importantly, to increases in such physiologically active lipid fractions as phospholipids and sterols.

Thus, the perspectivity of the *Streptomyces fradiae* CNMN-Ac-11 strain was demonstrated. When grown on complex nutrient media the strain can accumulate enough biomass with high content of lipids, including such physiologically important fractions as phospholipids and sterols.

*The research was funded by NARD within project 20.80009.7007.09.*

**PROMISING MICROORGANISMS FOR TREATMENT OF POULTRY PROCESSING WASTEWATER**

Hubchyk K., Hlushen A., Birukou R.
*Institute of Microbiology of the National Academy of Sciences of Belarus, Belarus*
e-mail: *gem@mbio.bas-net.by*

CZU:628.387.3 https://doi.org/10.52757/imb22.20

Massive volumes of effluents discharged by the poultry-processing plants contain elevated levels of pollutants (dissolved keratins, blood, lipids and proteins), and are distinguished by high BOD and COD values, as well as by large concentrations of suspended particles [1]. Therefore, there is an urgent need to develop effective and eco-safe methods protecting the environment from contamination. Among such methods the application of efficient microbial degraders of toxicants, possessing enhanced flocculating and decomposing activity, that can be introduced into the decontamination units to supplement the microbiota of the activated sludge at various stages of treatment, seems to be especially promising. Currently, biopreparations of microbial consortia intended for remediation of the environments exposed to organic pollutants are widely spread. The most common constituents of such consortia represent such genera as *Rhodococcus*, *Bacillus*, *Pseudomonas*, *Arthrobacter*, etc. [2].

Our study was focused on the microorganisms of the *Rhodococcus* and *Bacillus* genera, which are able to utilize the organic compounds in the poultry processing wastewater. There were selected 14 variants out of 145 screened strains that were either deposited in the laboratory of environmental biotechnologies, Institute of Microbiology, NAS of Belarus, or isolated from effluents of poultry-processing plants. They were tested for the COD degradation activity in poultry-processing wastewater, assayed for the proteolytic and lipolytic activities [3], and estimated for the biosurfactant forming potential [4].

The degradation activity of the selected microbial cultures was investigated using poultry-processing effluents with initial COD value of 1144 $O^2$/$dm^3$. It was found that the top COD reduction was by *B. subtilis* 6/2-APF1, *B. coagulans* 1710, *Bacillus* sp. FL-9MV, *Bacillus* sp. FL X-5, *Bacillus* sp. PF1, *R. ruber* 30P, *R. ruber* 200N, *R. ruber* 1NG – 52,3-71,6%. The maximum proteolytic activity toward milk proteins was observed for *B. coagulans* 1710, *B. subtilis* 6/2-APF1, *Bacillus* sp. FL-9MV, *Bacillus* sp. FL X-5. The lipolytic activity on the tributyrin-containing medium was detected in all 14 tested strains – B. *coagulans* 1710, *B. subtilis* 6/2-APF1, *Bacillus* sp. FL-9MV, *Bacillus* sp. FL X-5, *Bacillus* sp. PF1, *R. erythropolis* 7D, *R. erythropolis* 23F, *R. erythropolis* 70F, *R. ruber* 2B, *R. ruber* 1NG, *R. ruber* 30P, *Rhodococcus* sp. P1, *Rhodococcus* sp. G13, *R. ruber* 200N with the last two being especially active. The highest capacity to produce biosurfactants in the specific nutrient medium with methylene blue was displayed by *B. coagulans* 1710, *B. subtilis* 6/2-APF1, *Bacillus* sp. FL-9MV, *Bacillus* sp. FL X-5. In addition, the ability to produce surface active agents was observed for the bacterial cultures of *Bacillus* sp. PF1, *R. ruber* 2B, *Rhodococcus* sp. R1-3FN, *Rhodococcus* sp. G13 and *R. erythropolis* 7D.

Among the tested bacterial strains, the following cultures proved to be the most effective in decontamination of the poultry-processing wastewater: *B. coagulans* 1710, *B. subtilis* 6/2-APF1, *Bacillus* sp. FL-9MV, *Bacillus* sp. FL X-5, *Bacillus* sp. PF1. The above-listed microorganisms may act as promising components of the biopreparations promoting decontamination of the poultry-processing effluents.

## References:

1. Terán Hilares, R. Promising physicochemical technologies for poultry slaughterhouse wastewater treatment: a critical review / R. Terán Hilares [et al.] // Journal of Environmental Chemical Engineering. – 2021. – Vol. 9, № 2. – P. 105174.
2. Yagafarova, G.G. Features of the process of activation of native microflora for cleaning the soil from ecotoxicants / G.G. Yagafarova // Bulletin of the Technological University. – 2016. – Vol. 19, № 11. – C. 205-207.
3. Capodifoglio, E. Lipolytic and proteolytic activity of Pseudomonas spp. isolated during milking and storage of refrigerated raw milk / E. Capodifoglio [et al.] // Journal of Dairy Science. – 2016. – Vol. 99, № 7. – P. 5214-5223.
4. Biosurfactants: production and applications / R.S. Reis [et al.] // Biodegradation – Life of Science / ed. R. Chamy. – InTech, 2013. – Biosurfactants.

**THE EFFECT OF FARMING SYSTEM ON SOIL PROKARYOTIC COMMUNITIES IN MOLDOVA**

Indoitu D.
*Institute of Microbiology and Biotechnology, Moldova*
e-mail: diana.indoitu@imb.md

CZU:579.64+631.461 https://doi.org/10.52757/imb22.21

The soil microbiome plays an important role in the functioning of ecosystems. The influence of farming systems on the soil prokaryotic communities has not been sufficiently studied. Using metagenomic analyzes (high-throughput sequencing of the 16S rRNA gene on the Illumina platform), we studied the abundance, structure, and diversity of the prokaryotic community of the typical low-humus chernozem in two forage crops rotations (with and without inclusion of alfalfa) on the following variants: control without fertilizers (CON), mineral fertilizers (NPK) and organic fertilizers - manure (ORG). The research was carried out in the long-term field experiment on the "Biotron" Experimental Station of the Academy of Sciences of Moldova (Chisinau). The doses of fertilizers were calculated according to the crop in order to compensate for the quantitative content of NPK used, 100 tons of manure instead of $N_{450}P_{109}K_{470}$. Soil samples were taken from the top soil layer (0-30 cm) in 2021. The metagenomic analysis of soil was done using equipment of the Core Centrum 'Genomic Technologies, Proteomics and Cell Biology' in ARRIAM. This research was conducted as part of state projects of the Republic of Moldova 20.80009.5107 "Efficient use of soil resources and microbial diversity through the use of elements of biological (organic) farming".

Analysis of the soil prokaryotic communities in typical chernozem of the Central Zone of Moldova revealed the dominant presence of 5 phyla of bacteria in 2020 and 4 phyla of bacteria in 2021: *Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, *Firmicutes* and *Acidobacteriota* (in 2020) (table 1). Only one of identified phyla belongs to the archaea – *Nitrososphaerota* (*Thaumarchaeota)*.

**Table 1. The dominant phyla, 2021**

| | **Crop rotation with alfalfa** | | | | | |
|---|---|---|---|---|---|---|
| | CON | | NPK | | ORG | |
| 1 | ***Proteobacteria*** | **14,67%** | ***Thaumarchaeota*** | **15,32%** | ***Proteobacteria*** | **11,64%** |
| 2 | ***Actinobacteriota*** | **14,04%** | ***Actinobacteriota*** | **14,04%** | ***Thaumarchaeota*** | **11,33%** |
| 3 | ***Thaumarchaeota*** | **10,39%** | ***Proteobacteria*** | **10,81%** | ***Actinobacteriota*** | **8,90%** |
| 5 | ***Bacteroidota*** | **6,84%** | ***Firmicutes*** | **4,56%** | ***Bacteroidota*** | **4,33%** |
| 6 | ***Firmicutes*** | **4,00%** | ***Bacteroidota*** | **2,46%** | ***Firmicutes*** | **3,05%** |
| | **Crop rotation without alfalfa** | | | | | |
| 1 | ***Proteobacteria*** | **13,00%** | ***Actinobacteriota*** | **14,62%** | ***Thaumarchaeota*** | **16,23%** |
| 2 | ***Thaumarchaeota*** | **12,99%** | ***Thaumarchaeota*** | **13,36%** | ***Actinobacteriota*** | **10,14%** |
| 3 | ***Actinobacteriota*** | **11,92%** | ***Proteobacteria*** | **12,02%** | ***Proteobacteria*** | **7,08%** |
| 5 | ***Bacteroidota*** | **4,18%** | ***Firmicutes*** | **4,10%** | ***Firmicutes*** | **3,94%** |
| 6 | ***Firmicutes*** | **3,46%** | ***Bacteroidota*** | **3,24%** | ***Neclasificat Bacteria*** | **1,94%** |

*Proteobacteria* and *Actinobacteriota* dominated in all studied variants and in all studied years. The content of *Actinobacteriota* was slightly lower in the rotation with alfalfa in all studied variants. The content of *Bacteroidota* was higher in the crop rotation without alfalfa in all variants. The most numerous family in 2020 was *Micrococcaceae* family from the phylum *Acidobacteriota*, then *Chitinophagaceae* from the phylum *Bacteroidota* and *Sphingomonadaceae* from the phylum *Proteobacteria*.

*This research was carried out within the State Program Project 20.80009.5107 (2020-2023), financed by ANCD.*

# SOME FEATURES OF CULTIVATION OF THE ACTINOBACTERIUM *SACCHAROPOLYSPORA SPINOSA*

Lungu A.
*Institute of Genetics, Physiology and Plant Protection, Republic of Moldova*
e-mail: andrei.lungu@igfpp.md

CZU:579.64:631.46 https://doi.org/10.52757/imb22.22

**Keywords:** actinobacteria, *Saccharopolyspora spinosa*, spinosad, cultivation, strain DSM-44228.

Purpose: Actinobacteria (actinomycetes) are in the center of attention because these bacteria produce a variety of natural drugs and other biologically active metabolites, including: antibiotics, enzymes, inhibitors. More than 22,000 biologically active secondary metabolites (including antibiotics) produced by microorganisms have been identified and published in the literature and patented. About half of these compounds are produced by actinomycetes. Currently, about 160 antibiotics are used in medicine and agriculture, 100-120 of these compounds, including streptomycin, erythromycin, gentamicin, vancomycin, vermectin, etc. are produced by actinomycetes. However, the use of actinomycetes for the development of new methods and means is increasingly difficult. Although a large number of microorganisms have been identified, described, verified, more than 90% of all microorganisms remain unutilized. These species could be used intensively to obtain new means, which would contribute to a sustainable development of human society [D. Dhanasekaran, 2016].

Spinosins are new macrolides, natural metabolites produced under aerial fermentation conditions by the species *Saccharopolyspora spinosa*. These compounds contain a unique system of tetracyclic rings to which two sugar residues are attached. Spinosad, a mixture of spinosyns A and D, is used as a unique pesticide with high selective activity against target pests and low toxicity in non-target organisms (including many beneficial arthropods). These characteristics make spinosad a good new tool for integrated pest management. The discovery and characterization of *S. spinosa* represents a new opportunity to develop progressive insect management tools using native products [MERTZ, and YAO, 1990], [Guojun Y, 2016].

Materials and Method: The strain *Saccharopolyspora spinosa* DSM-44228 was used for the experiments. Cultivation was carried out in the initial stages on agar medium and then moved to deep cultivation on liquid medium. We developed and used several compositions of the liquid medium, quantitative changes were made to the components and it was used several sources of carbon and nitrogen. In the same way, the cultivation time, temperature, pH of the culture medium was changed in all variants, the rocking shaker has 150 r/m. As a carbon source, it was used glucose, sucrose, and maltose. As an alternative source it was used also soy, corn, and sunflower flour.

Results: So far we have been able to achieve good growth and sporulation over 7 days on agar medium. On liquid medium we have developed two compositions on which there was a good accumulation of biomass, but they have not yet been determined the amount of produced Spinosad. We are going to carry out the optimization of the medium to achieve a maximum possible production of biomass.

**References:**

D. Dhanasekaran, Y. Jiang (Eds.) Actinobacteria - Basics and Biotechnological Applications. London, United Kingdom, IntechOpen, 2016 [Online]. Available from: https://www.intechopen.com/books/5056 doi: 10.5772/60457

Guojun Y, Yuping H, Yan J, Kaichun L, Haiyang X. A New Medium for Improving Spinosad Production by *Saccharopolyspora spinosa*. Jundishapur J Microbiol. 2016 May 30;9(6): e16765. doi: 10.5812/jjm.16765. PMID: 27635207; PMCID: PMC5013548.

MERTZ, E.P.; YAO, R.C.; *Saccharopolyspora spinosa* sp. isolated from soil collected in a sugar rum still. International Journal of Systemic Bacteriology. 1990, p. 34-39.

*Acknowledgment: Research was carried out within the project of the State Program 20.80009.7007.04 "Biotechnologies and genetical processes for evaluation, conservation and exploitation of agrobiodiversity", financed by the National Agency for Research and Development. We thank Leibniz Institute, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH for granting S. spinosa strain DSM 44228.*

**STUDY OF THE ADSORPTION OF *BACILLUS SUBTILIS* ON DIFFERENT FRACTIONS OF ACTIVATED CARBONS OBTAINED FROM APPLE WOOD**

Lupascu L., Petuhov L., Lupascu T., Timbaliuc N.
*Institute of Chemistry, Ministry of Education and Research, Republic of Moldova*
e-mail: lucianlupascu75@gmail.com

CZU:579.254.4+632.934.14 https://doi.org/10.52757/imb22.23

The paper presents the results of scientific research related to the study of adsorption processes of *Bacillus subtilis* bacteria on activated carbon obtained from apple wood. The activated carbons used have a specific BET area of about 2018 $m^2/g$ and a total sorption volume of the pores equal to 1,573 $cm^3/g$. The study of the kinetics of the adsorption processes of the *Bacillus subtilis* bacteria showed that the value of the maximum adsorption for the fraction 630-800 μm is established within 90 min. The maximum adsorption capacity is 0,54-0,55 McF*$10^8$/g, higher than activated charcoal obtained from apricot stones that has the values of 0,375-0,385 at 27°C. The peak of the adsorption is noticed after 90 minutes of the contact and is presented in the figure bellow:

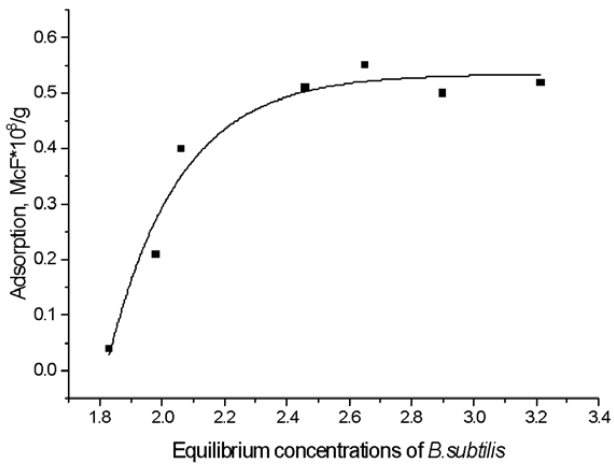



**Figure 1.** Adsorption isotherm of *B. subtilis* bacterium on AC-apple, fraction 630-800 μm at 27°C

The kinetics of the adsorption processes of activated carbon obtained from apple wood (fraction 800-2000 μm) for the same bacteria were evaluated at 27°C. The main sorptive properties were attested after 90 to 120 minutes of contact. The adsorption values are in the range of 0,21-0,25 McF*$10^8$/g depending on the contact time, lower than in the case of the fraction 630-800 μm and are approximately at the level of activated carbons obtained from apricot stones tested at 37°C. The peak of the adsorption is noticed after 120 minutes of contact. We can conclude that the sorption capacity of the activated carbon of the fraction 630-800 μm obtained from apple wood is 2 times higher than in the case of the 800-2000 μm fraction of the same carbons for the *B. subtilis* bacterial species.

*Acknowledgement: This research was carried out with the financial support of the institutional project "The reduction of the environmental and health impact of toxic chemicals through use of adsorbents and catalysts obtained from local raw material" DISTOX, No 20.80009.7007.21*

**GROWTH AND SPORULATION OF *BEAUVERIA BASSIANA* ON DIFFERENT CULTURE MEDIA**

Moldovan A.[1,2], Ivantoc N.[2], Munteanu-Molotievskiy N.[1]
[1]*Institute of Zoology, Republic of Moldova*
[2]*Moldova State University, Republic of Moldova*
e-mail: anna.moldovan@yahoo.com

CZU:632.937.14 https://doi.org/10.52757/imb22.24

The entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill. 1912 (Hypocreales: Cordycipitaceae), thanks to its effective insecticidal properties, is widely used as a biological control agent. A critical issue in the mass-production of fungal-based biopesticides is the selection of the culture media optimal for the growth and sporulation of the producer strain. The most often used medium for the cultivation of *B. bassiana* is Potato Dextrose Agar (PDA). However, the relatively high price of the commercially available PDA medium determines the need to look for an alternative culture medium that would allow efficient and profitable cultivation of entomopathogenic strains for industrial purposes. Thus, the research aimed to select the optimal nutrient medium for mass-production of a local *B. bassiana* strain (Invention Patent MD 4560).

The *B. bassiana* CNMN-FE-01 strain's vegetative growth was studied on several nutrient media for 14 days at a constant temperature of 25°C. In addition, the number of conidia produced per unit area and the germination rate of these conidia after cultivation on each media were determined. Of all analyzed media three represented commercially available formulations (PDA, SDA - Sabouraud Dextrose Agar, and SAPF - Selective Agar for Pathogenic Fungi), and two were readymade formulations supplemented with yeast extract (SDAY and PDAY). The other were prepared in the laboratory according to known recipes (PSA - Potato Sucrose Agar, PDA, PDAY, Oatmeal Agar).

As a result, *B. bassiana* CNMN-FE-01 strain can be successfully cultivated on various solid media, commercially available formulations, and media prepared in the laboratory. The maximum radial growth rate of the micromycete was recorded on the Oatmeal Agar medium. Also, on Oatmeal Agar, the local strain produces the highest number of viable conidia. The experimental data indicate that the micromycete growth rate differs depending on the culture medium used. The same culture medium but from different producers can induce distinct growth and sporulation patterns of the fungal strain. The current work emphasizes the necessity to verify the purchased media and to identify a simple, cost-efficient media that can be easily prepared on site but also suggests the importance of setting the quality control points in the mass production of fungal-based biopesticides.

*The presented scientific results were financially supported by the project 20.80009.7007.12 "Diversity of hematophagous arthropods, zoo - and phytohelminths, vulnerability, strategies to tolerate climatic factors and the elaboration of innovative procedures for the integrated control of species with socio-economic value" – DIVZOO.*

**DESTRUCTIVE ACTIVITY OF MICROORGANISMS IN RELATION TO GLYCOL ETHERS**

Narkevich D., Hlushen A.
*Institute of Microbiology NAS Belarus, Minsk, Belarus*
e-mail: dasha.narkevich@mail.ru

CZU:579.64+632+631.95 https://doi.org/10.52757/imb22.25

Glycol ethers, especially 2-butoxyethanol (BE) and 2-ethoxyethanol (EE), have low human toxicity and are widely used as components of paints and solvents, inks, detergents, liquid soaps, hydraulic fluids, etc. [1]. The high ability of glycol ethers to dissolve in water due to their amphiphilic structure ensures their ingress and accumulation in aqueous systems. Currently, there is limited literature data on the bacterial degradation of these compounds, which makes this an urgent problem to study [2, 3].

Seventeen cultures were previously selected which showed the ability to utilize glycol esters at a concentration of 0.5%. These strains were tested for their ability to degrade glycol ethers in the concentration range from 0.1% to 3%. It was found that 2-butoxyethanol is a preferred carbon source compared to 2-ethoxyethanol.

It is known that the process of degradation of complex organic substances by microorganisms often depends on the presence of calcium and iron ions in the medium, which are part of the enzyme systems involved in the processes of biodegradation of xenobiotics. In connection with this fact, we studied the utilization of glycol ethers in different mineral media containing calcium and iron ions. It was found that the presence of iron ions in the mineral medium significantly intensifies the process of degradation of toxicants in the following strains – *Rhodococcus* sp. VOC 5, *Rhodococcus* sp. VOC 14, *Rhodococcus* sp. VOC 8/7. The rate of glycol ester utilization by cultures of *Rhodococcus* sp. CLV-2, *Rhodococcus* sp. SCV-1, *Rhodococcus* sp. SCV-2 and unclassified strain JD 4.14 were independent of the presence of calcium or iron ions in the medium, which suggests their promising use for the development of a microbial preparation for glycol ester wastewater treatment.

The destructive activity of the selected strains against 2-butoxyethanol was evaluated. The experiment was carried out in flasks containing tap water, iron salts and glycol ether at a concentration of 0.05% as the only carbon source. This concentration was chosen because the most common concentration of glycol ether in wastewater was 100 – 600 mg/l. As a result of these studies, it was shown that the culture of *Rhodococcus* sp. VOC-5 and unclassified strain JD 4.1 most actively utilize 2-butoxyethanol. During cultivation of *Rhodococcus* sp. VOC-5, a change in the odor of glycol ether to the odor of its putative decomposition products (aldehydes and acetates) was noted, indicating the processes of its degradation by microorganisms. The unclassified strain JD 4.1 exhibited more than 90% of its original degradation activity against 2-butoxyethanol in the test solution.

**References:**

1. 2-butoxyethanol: Risk assessment, European Union Risk Assessment Report / eds. P. Copestake [et al.] // Geneva: WHO. – 2009. – T. 67. – 60 p.

2. Biology of triacylglycerol accumulation by *Rhodococcus* / H. M. Alvarez [et al.] //Biology of *Rhodococcus*. – Springer, Cham. – 2019. – P. 299-332.

3. Biodegradation of ethyl t -butyl ether (ETBE), methyl t -butyl ether (MTBE) and t -amyl methyl ether (TAME) by Gordonia terrae / G. Hernandez-Perez [et al] // Applied Microbiology and Biotechnology. – 2001. – T. 55. – №1. – P. 117–121.

# THE EFFECT OF A FERTILIZER ON THE ABUNDANCE OF MICROORGANISMS IN SOILS SUBJECTED TO REMEDIATION

Postolachi O.[1], Rastimesina I.[1], Vorona V[1]., Bogdevich O.[2]
[1]*Institute of Microbiology and Biotechnology, Republic of Moldova*
[2]*Institute of Chemistry, Republic of Moldova*
e-mail: oleseap@yahoo.com

CZU:631.4 https://doi.org/10.52757/imb22.26

An important factor, which often limits the effectiveness of current biodegradation techniques, is the poor ability of the microbial communities inhabiting the contaminated environments to degrade the pollutants. Therefore, addition of fertilizers of organic and mineral origin, which trigger the metabolic processes of microorganisms and, as a result, stimulate their growth, is a common practice. The aim of our research was to evaluate the effect of a fertilizer on the abundance of microorganisms during soil remediation.

The object of study was a soil polluted by a POP complex, that was collected from the territory of a former pesticide storage, located near the village of Slobozia-Dușca, the Criuleni district, the Republic of Moldova. The bioremediation experiments were done in plastic jars, each containing 1,000 g of contaminated soil. The contaminated soil without remediation treatments was used as a control. The experiments were conducted under oxic (Variant 1) and cycled anoxic/oxic conditions (Variants 2-4). Under the oxic conditions the soil moisture was kept constant at 60%. The anaerobic conditions were created by saturating the contaminated soil with water at 80% of the water holding capacity in plastic jars sealed with black polyethylene. The bioremediation experiments were focused on stimulating the indigenous soil microflora by applying a fertilizer. The tested fertilizer contained 50.0% of wood sawdust, 40.0% of iron filings, 10.0% of organic compounds, and was introduced in the amounts of 3.0% (Variant 3) and 6.0% (Variant 4). Thus, for the bioremediation of the polluted soil, there were in total 4 experimental variants. The dynamics of the microbial population in the soil subjected to bioremediation was evaluated during five cycles by the spread plate method. Estimating the total number of microorganisms in the soil samples after 5 cycles of bioremediation showed that the addition of fertilizer significantly stimulated the growth of microorganisms. Most actively the microbial population grew in variant 4 (Figure). Comparing to the polluted soil control, the population of microorganisms in Variant 4 increased by 155 times, and comparing to Variant 1 – by 25.4 times. The remediation by cycled oxic-anoxic conditions (Variant 2) did not have considerable effects on the microorganisms, while the addition of the fertilizer (Variants 3 and 4), under the same conditions, significantly stimulated their abundance.

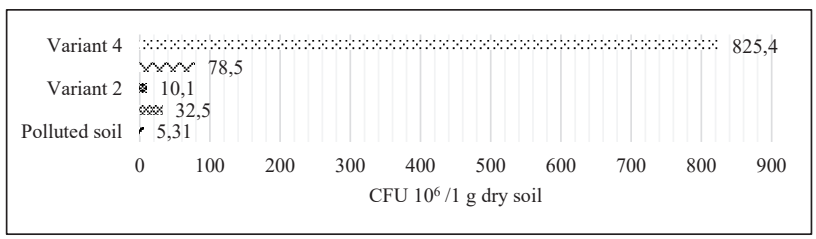



**Figure 1**. The total number of microorganisms in the soil samples after five cycles of bioremediation

Thus, it was established that the tested fertilizer can serve as a bioremediation factor in cyclic anoxic/oxic conditions, stimulating the development of the microbial community in the soil contaminated by POP complexes. Under the conditions of our experiment the best fertilizer concentration was 6.0%.

*This work was funded by NARD of the Republic of Moldova within State Program Project 20.80009.7007.20 "Study and management of pollution source for the elaboration of recommendations for implementing to reduce the negative impact on the environment and population health".*

# THE INFLUENCE OF NANOMAGNETITE ON THE PROCESSES OF GROWTH, DEVELOPMENT, AND FORMATION OF THE LEGUME-RHIZOBIA COMPLEX IN VETCH PLANTS UNDER SOIL CONDITIONS OF PLASTICS POLLUTION

Prisacari S., Todiras V., Corcimaru S.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: prisacarisvetlana@rambler.ru

CZU:[579.64+631.461]:57.08 https://doi.org/10.52757/imb22.27

The Republic of Moldova suffers from the problem of environmental pollution by plastics, including by the low-density polyethylene (LDPE). The accumulation of plastics by plants has negative consequences for the food security and sustainable development of the agriculture. It is suggested that over time soil pollution by plastics can threaten the successful functioning of the entire agricultural system.

The negative consequences of soil pollution by plastics impose the need of developing measures of remediation. Due to the lack of efficient chemical and physical methods for destroying plastics in soil, the attention has recently been directed toward developing biological degradation techniques, including the ones based on application of phytoremediation and nanophytoremediation. However, the potential of these techniques in the cases of soil pollution by LDPE is understudied. The aim of this work was to explore the possibility of using nanomagnetite and vetch plants bacterized by the *Rhizobium leguminosarum* K2 strain for remediation of soils contaminated by LDPE.

The introduction into soil of a finely chopped LDPE (5 g/kg) and nanomagnetite (25 mg/kg of soil) resulted in increases in the total length of plants (roots included), plant height, and the accumulation of dry biomass of 10.6%, 15.4%, and 28.8% respectively. The number of root nodules was higher by 2.2 times. Positive effects were also observed in the two variants where LDPE was introduced without nanomagnetite and the vetch seeds were either inoculated or not inoculated by rhizobia. Comparing to the control, the root length, plant height, and dry mass had 8.2%, 11.7%, and 26, 8% increases respectively. The number of root nodules in these variants was 2.4–2.8 times higher than in the control.

Even though not all effects were significant statistically, the general picture showed that the introduction of LDPE into soil had no inhibitory effects on plant productivity and formation of the legume-rhizobia complex, and even stimulated them, especially in the cases of seed inoculation by rhizobia and nanomagnetite treatment.

The observed formation of healthy legume-rhizobia complexes in the variants where the plant seeds were inoculated by *Rhizobium leguminosarum K2* is of a significant importance for plant productivity, as well as for soil fertility. Rhizobia within this symbiosis provide the plants with the nitrogen fixed from the atmosphere, and, in turn, obtain from them the needed organic substrates. It is known that due to the symbiotic nitrogen fixation, the soil annually can receive up to 90-180 kg/ha of nitrogen. The observed stimulation of dry mass accumulation was important too. The fact that the plants and the symbiosis with rhizobia could be stimulated in the presence of LDPE contamination demonstrated the possibility of using the vetch plants as an efficient phytoremediator in cases of soil pollution by plastic waste.

*The presented data were obtained within the research project "Microbial tools for degradation of non-recyclable plastics waste", registered under code 20.80009.7007.03 in the State Program for 2020-2023, funded by the National Agency for Scientific Research and Development of the Republic of Moldova.*

**IDENTIFICATION OF MIXED MICROBIAL CONSORTIA ISOLATED FROM POLYETHYLENE FILMS SURFACE**

Rastimesina I., Postolachi O., Vorona V., Mamaliga V., Voinescu A.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: rastimesina@gmail.com

CZU:606:579.6 https://doi.org/10.52757/imb22.28

Polyethylene, a highly recalcitrant and inert synthetic material and thereby very difficult to degrade in the environment has become a major source of environmental pollution. The traditional methods for plastic waste dispose are recycling, incineration, and landfilling, the oldest and most common method of plastic waste disposal. The natural degradation of low-density polyethylene (LDPE, PE-LD) depends on polymer properties, its high molecular weight, hydrophobic nature and lack of functional groups, recognized by microbial enzymes makes it adverse towards degradation. However, several studies have reported the polyethylene degrading microorganisms isolated from waste disposal sites. Various species of bacteria and fungi or microbial communities, isolated from soil, are able to modify and consume the plastic polymers as a source of energy.

The purpose of present study was to characterized microbial consortia isolated from the surface of LDPE films extracted from the soil contaminated with polyethylene.

LDPE films were placed in the soil that was collected from the landfill, located near the village of Slobozia-Duşca, the Criuleni district, the Republic of Moldova. The soil was treated under aerobic and anaerobic conditions within six months.

Fungal and bacterial strains were isolated from LDPE surface, through enrichment techniques. Enrichment cultures were prepared by adding 10 mL water sample to 90 mL mineral salt medium (MSM). At the initial stage of creating consortia in the culture media was added LDPE in the form of granules, in an amount of 1 g. As a growth inducer in the media was added glucose, in a concentration of 0.1 mL. Samples from the enrichment culture were serially diluted and plated onto MSM agar, nutrient agar, Czapek medium. Bacterial isolates were then allowed to grow by incubating the plates at 28°C for five days. Growing colonies were selected and streaked successively onto the same media for purification. The isolates were examined for their Gram reaction, endospore formation, and cultural characteristics, such as colour, colony form, margin, surface, and elevation.

The data obtained show that after 100 days of cultivation the microorganisms in the consortia retain their viability, the titer being from $7.00 \times 10^6$ CFU/mL, up to $32.00 \times 10^6$ CFU/mL. The consortia obtained are composed predominantly of fungal strains, and micromycetes are mostly represented by the genus *Trichoderma*. The bacteria were determined only in 2 consortia, out of the 6 obtained, and were represented by species from the genera *Bacillus*, *Pseudomonas*, *Streptomyces*.

*Acknowledgments. This work was funded by NARD of the Republic of Moldova within State Program Project 20.80009.7007.03 "Microbial tools for degradation of non-recyclable plastics waste."*

**EFFECT OF PHAGES ISOLATED FROM DIFFERENT SOURCES AGAINST FIRE BLIGHT PATHOGEN**

Samoilova A.
*Institute of Genetics, Physiology and Plant Protection*, *Republic of Moldova*
e-mail: anna.v.samoilova@gmail.com

CZU:632.937.16 https://doi.org/10.52757/imb22.29

Fire blight of rosaceous plants is one of the economically most important diseases of fruit trees caused by the bacterium *Erwinia amylovora*. Plants are extremely vulnerable for fire blight infection at the bloom stage. Blossom blight can lead to the great crop losses and even the plant death. Since chemical treatments are forbidden in time of blossoming, bacteriophages, highly specific bacterial viruses could be used for the disease control. Being the natural components of ecosystems, phages infect only bacteria sensitive to them, are non-toxic to plants, animals and humans and are adapted to the bacteria environment.

It has been shown that bacterium *E. amylovora* expresses its major pathogenicity factors during immature pear tissues infection. Therefore, in this study, the ability of four virulent *E. amylovora* bacteriophages, isolated from the aerial parts of the affected plants (phage isolate 1 from quince tissues; phage isolate 2 from hawthorn, Republic of Moldova) and from natural water reservoirs near fruits orchards or wild rosaceous trees (phage isolates 3 and 4, Swiss Confederation) to inhibit *E. amylovora* growth in the immature pear tissues was evaluated.

Immature pear slices were inoculated with suspensions of *E. amylovora* CFBP1430 and EaM contained $10^8$ CFU/ml. After four hours incubation in the humidified chamber at 28°C infected immature pear slices were treated with $10^7$ PFU/ml of phage isolates. Pear slices, treated with sterile distilled water were used as a control. Symptoms were recorded at 1, 2, 3, 5, 6, 7 and 8 days after inoculation. For each bacteria strain/phage isolate combination tested pear slices were assayed in triplicate and each experiment was repeated at least two times.

Immature pear slices infected with bacteria EaM displayed the first symptoms of the fire blight, ooze formation and light necrosis, one day after inoculation. Pear slices, infected with *E. amylovora* CFBP1430 demonstrated ooze and necrosis two days after inoculation.

In the bacteria/phage combinations the first symptoms of the fire blight appeared on the sixth day after inoculation in the variants of EaM/phage isolate 3 and CFBP1430/phage isolate 3. On the seventh and eighth days after inoculation symptoms of the fire blight infection have been recorded in the EaM/phage isolate 2 and CFBP1430/ phage isolate 2, respectively. Bacteria/phage combinations EaM/phage isolate 4 and CFBP1430/ phage isolate 4 showed disease symptoms on the seventh day after inoculation. Immature pear slices in the variants EaM/phage isolate 1 and CFBP1430/phage isolate 1 showed necrotic lesion eight days after inoculation. Thus, phage isolate 4, detected in water was able to suppress growth of phytopathogenic *E. amylovora* just a day less than highly virulent phage isolate 1 detected in the quince tissues.

The conducted experiments have demonstrated that bacteriophages isolated from water revealed high efficacy against bacteria *E. amylovora* and all studied phage isolates successfully inhibited the fire blight causative agent growth in the plant host tissues for about seven days. Hence it has been shown that treatment with bacteriophages for the fire blight control in the fruit orchard should be carried out weekly if environmental conditions are favorable for the disease development.

*Research was carried out within the Swiss Government Excellence Postdoctoral Scholarships for Foreign researchers and project of the State Program 20.80009.7007.16 "Synergy between natural factors and environmentally friendly microbiological means of pest population density regulation for crop protection in conventional and organic agriculture", financed by the National Agency for Research and Development.*

# NEW SELENIUM-ENRICHED FODDER YEASTS AND THEIR APPLICATION IN RATIONS OF LAYING HENS

Sapunova L.[1], Moroz I.[1], Pauliuk A.[1], Romashko A.[2], Senko A.[2]

[1]*Institute of Microbiology, NAS Belarus, Minsk, Belarus*
[2]*Experimental scientific station of poultry breeding, Zaslavl, Belarus*
e-mail: leonida@mbio.bas-net.by

CZU:636.52/.58.087.7 https://doi.org/10.52757/imb22.30

## Introduction

The growing demand for selenium-containing food and folder is determined by its role to provide normal life activities of all biological creatures, including humans and animals. Selenium (Se) normalizes liver function, shows antioxidant, immunomodulating, detoxifying properties, is actively involved in generation of active sites in several enzymes. The lack of selenium is responsible for metabolic disbalance, retarded growth, degenerative changes in muscle and hepatic tissues, cardiomyopathy, reproductive dysfunction. The shortage of this microelement on the cellular level disrupts integrity of cell membranes, reduces enzyme activity, provokes intracellular calcium accumulation, impairs metabolism of amino- and ketoacids, suppresses energy generation processes, etc. This, in turn, causes such pathologies as cancer, Keshan or Kashin-Beck diseases, muscular syndrome, often resulting in lethal outcome [1–4].

Alimentary products of plant and animal origin as well as water serve as sources of selenium for humans and animals. Therefore, depending on the geochemical factor, the content of the microelement in the consumed food and feed products determines the level of its physiological uptake by humans and animals. Taking into account that selenium consumption dose recommended by World Health Organization varies from 40 to 200 μg per day, its deficiency on the global scale affects 0.5 to 1.0 bln people. Belarus is distinguished by the moderate selenium deficit requiring permanent supply of Se-enriched premixes. Their introduction into feed rations solves the problem of microelement deficiency not only in animals, but also in the human body through the consumption of animal products enriched with this microelement.

Inorganic selenium widely used to date in feed formulas is not efficiently assimilated, displays low biological value, high toxicity, in overdoses may induce toxicoses or even lethal cases. On the contrary, organic selenium compounds are less toxic, hence they are more suitable for incorporation into tissues [5–6]. The most readily available, economically grounded and ecologically safe method of commercial production envisages transformation of inorganic selenium in microbial cells into organic Se varieties.

Among selenium-accumulating microorganisms, bacteria of genera *Lactobacillus*, *Propionibacterium*, *Bacillus*, mycelial fungi of genus *Aspergillus*, basidiomycetes of genera *Ganoderma*, *Agaricus*, *Grifola*, *Hericium* and yeast-like fungi may be pointed out.

The latter are represented by natural selenium-adapted cultures *Candida utilis* CUM, *Hanseniaspora uvarum* (*Hansenula vinifera*), *Saccharomyces cerevisiae* TZJM and mutant strains subjected to chemical and physical mutagenesis (*Candida glabrata* FXY-4, *Candida utilis* S1204), as well as molecular genetic modifications (*Rhodotorula glutinis* X20).

The world market of Se-containing yeast abounds in plenty of manufacturers dominated by the most influential corporate players, like Assotiated British Foods Inc. (UK), Archer Daniels Midland Company (US), Alltech Inc. (US), Cargill (US), Angel Yeast Company (China), Chr. Hansen (Denmark), Lesaffre (France), etc. In the countries of Eurasian Economic Community microbiological manufacturing of selenium-enriched products has not been launched so far. Currently the project aimed at elaboration of pilot-plant technology of producing Se-enriched fodder yeasts based on strain *Candida stellimalicola* 4-ASe adapted to this microelement is nearing the completion at the Institute of Microbiology, National Academy of Sciences of Belarus [7].

The aim of the present investigation is the comparative evaluation of the effect of sodium selenite and Se-enriched fodder yeasts on productivity of laying hens and egg quality.

**Materials and methods**

Feed yeast culture fortified with selenium in concentration 2,000 mg/kg, was based on strain *Candida stellimalicola* 4-ASe adapted to this microelement. The strain was deposited in Belarusian collection of non-pathogenic microorganisms under registration number BIM Y-350 D.

The studies were carried out at Genestock division of the 1st Minsk poultry factory and at Experimental research station for poultry breeding. To conduct experiments 3 groups of local cross-bred laying hens were chosen, each comprising 30 heads. The groups were formed in accordance with the principle of similarity in origin, age, gender, live weight. The fowl was kept in individual cages. Rearing density, light, temperature, humidity regimes, and other breeding parameters matched the standards set for the tested cross line.

Laying hens of the first control group were fed the composite ration balanced in major nutrients and sodium selenite (200 mg Se / t) added into standard premix formula. The fowl from 2 and 3 test groups was fed composite fodder fortified with Se-enriched yeast in amount 0.1 and 0.15 g/t (200 and 300 mg Se / t), respectively. The test batches of composite feed were fabricated by Alnikorprodukt Vertelishki (Belarus).

The generally recognized methods were used to determine survival of chickens, live body weight, fodder consumption, egg-laying capacity, egg weight, feed expense per 10 eggs, fodder spent to produce 1 kg of egg mass, the yield of egg mass per 1 fowl, egg category, morphological composition of eggs, the contents of vitamin A and carotenoids in egg yolk.

Statistical processing of experimental data was performed by regression analysis (Student's test) using Microsoft software.

**Results and discussion**

The results of feeding trials demonstrated that supply into the rations of both sodium selenite (200 mg Se / t) and selenium-fortified yeast (200 and 300 mg Se / t) did not affect viability and productivity of laying hens. In all experimental groups egg- laying capacity per average hen did not vary significantly: 56.6 eggs in the first control group versus 56.4 and 56.8 eggs in the second and third test groups. When selenium dose supplied into the rations with fodder yeast rose from 200 to 300 mg Se / t of composite feed egg productivity increased by 0.4%. The intensity of egg production in chickens of the 1 control group equaled 71.7%, whereas in the 2 and 3 groups it varied from 71.4 to 71.9%.

Irrespective of selenium source in the rations, the hens were eating the fodder readily and almost in the same amount. The average daily feed consumption in all groups was identical (126.9 g), as well as fodder expense (1.77–1.78 kg) per output of 10 eggs.

It should be noted, however, that Se-enriched yeast exerted stronger effect on the weight of produced eggs in comparison with added sodium selenite (Table 1)

**Table 1. The data of morphological egg examination**

| Parameters | Groups | | |
|---|---|---|---|
| | 1 (control) | 2 (test) | 3 (test) |
| Egg weight, g | 53.6±1.15 | 54.8±1.43 | 56.2±0.82 |
| Shape index | 76.1±0.50 | 76.2±0.54 | 76.9±0.48 |
| Haugh units | 77.9±2.40 | 79.9±4.80 | 83.0±2.18 |
| The ratio of albumen weight to yolk weight | 2.3±0.06 | 2.5±0.06 | 2.4±0.05 |
| Shell thickness, μm | 353±6.7 | 346±9.5 | 373±5.98* |
| Albumen index | 0.082±0.005 | 0.089±0.007 | 0.085±0.005 |
| Yolk index | 0.401±0.007 | 0.423±0.007* | 0.410±0.005 |
| Shell weight, g | 5.9±0.23 | 5.9±0.22 | 6.4±0.17 |
| Yolk weight, g | 14.4±0.30 | 14.3±0.39 | 14.9±0.26 |
| Albumen weight, g | 32.9±0.84 | 34.6±0.99 | 34.8±0.59 |

Note: *the difference between the parameters of the control and test groups is authentic at P≤0.01

In contrast to chickens from the first control group this parameter in hens of the 2 and 3 groups was higher by 2.9–3.5 % (P≤0.001). Likewise, the yield of the egg mass per each hen in the 2 and 3 groups was higher by 2.7–3.4% if compared with the control parameter. The increase of average egg weight in the 2 and

3 groups accompanied by the identical feed consumption resulted in the reduced feed expense (2.3–3.5%) to produce 1 kg of egg mass. The fact that substitution of organic selenium for inorganic analog in hen rations enhanced egg weight and saved fodder spent to produce 1 kg of egg mass was also stated by other researchers (8–9).

When sodium selenite was replaced with Se-enriched yeast in composite fodder, the rising tendency was recorded for the parameters characterizing egg incubation properties (table 1). For instance, eggs laid by the hens from 2 and 3 groups were distinguished by elevated Haugh units (2.6–6.5%), albumen index (3.7–8.5%), and yolk index (2.2–5.5%) ($P \leq 0.01$ for the 1 control and 2 test groups).

The similar ($P \leq 0.01$) beneficial effect of yeast fortified with selenium (300 mg Se / t) on thickness of egg shell was revealed in the 3-test group – this parameter equaled 373 μm; exceeding by 5.7% the similar parameter in hens of the 1 control group. As a result, egg shell mass in chickens of the 3-test group augmented by 8.5%. Favorable impact of organic Se compound in feed additive on morphological characteristics of eggs, namely on shell thickness was also recorded by other researchers [9].

The indexes of egg weight evaluated in the course of morphological examination and gravimetric assessment of gross egg yield were comparable in the 1 control and 2 and 3 test groups of laying hens. Noteworthy that weight of eggs produced by fowls in 2 and 3 test groups surpassed by 1.2–2.6 g that in the control group as a consequence of increased protein content in eggs by 5.2–5.8% (from 32.9g to 34.6–34.8 g) resulting in enhanced albumen to yolk mass ratio (from 2.3 to 2.4–2.5).

Upon supplementation of rations with Se-enriched yeast premixes concentration of vitamin A in eggs of hens from the 2 and 3 test groups rose by 1.7–2.4%, while the level of carotenoids tended to grow by 4.2–6.4% as compared with the control. The maximum amount of vitamin A and carotenoids was detected in eggs of poultry from the 2-test group receiving 200 mg Se / t of composite feed.

Distribution of eggs collected from fowl in all studied groups into quality categories is reflected in table 2.

**Table 2. Egg distribution into categories**

| Egg category | Egg distribution into categories, %: | | |
|---|---|---|---|
| | 1 control group | 2 test group | 3 test group |
| **Supreme** | 0 | 0.6 | 0.3 |
| **Premium** | 0.3 | 0.6 | 0.6 |
| **First** | 23.7 | 33.1 | 31.1 |
| **Second** | 68.9 | 64.8 | 65.0 |
| **Small** | 7.1 | 0.9 | 3.0 |

It is evident from table 2 a significant decline in the number of low-value eggs produced by the hens from the 2 and3 test groups: in comparison with the 1 control group the proportion of small eggs fell from 7.1 to 0.9–3.0%, the ratio of the eggs of the second category dropped from 68.9 to 64.8–65.0%. The share of eggs in top price categories increased considerably: the first category from 23.7 to 31.1–33.1%, premium category from 0.3 to 0.6%, supreme category from 0 to 0.3–0.6%.

Our findings are partially correlated with literature data testifying to the improved physical (survival rate, bodyweight gains, egg productivity) and reproductive (fertility, egg hatchability, puberty period) parameters of poultry, upgraded quality of farm products (enlarged weight of eggs, higher protein and selenium contents) depending on age, concentration and duration of Se-containing yeast supply [10–14].

**Conclusions**

Using a novel microbial strain *Candida stellimalicola* 4-ASe, the first in Belarus pilot-scale technology of manufacturing fodder yeast fortified with selenium was elaborated. A test batch of the new feed product containing 2000 mg Se / kg was produced and passed successful large-scale trials.

It was found that introduction into the rations of both sodium selenite and selenium-enriched yeast did not significantly affect viability and productivity of laying hens. However, addition into chiken feed formulas of selenium-enriched yeast (200 and 300 mg Se / t of composite fodder) in contrast to sodium selenite supplement (200 mg Se / t) improved morphological characteristics (Hough unis, albumen and yolk indexes, shell thickness) and raised protein ratio in eggs as well as concentrations of vitamin A and

carotenoids. Moreover, in laying hens nourished with selenium-enriched yeast, the yield of small and second category eggs tended to decrease with concomitant rise in the share of eggs representing the first (from 23.7 to 31.1–33.1%), premium (from 0.3 to 0.6%) and supreme (from 0 to 0.3–0.6%) categories.

Scaling up the process of manufacturing Se-containing feed additives based on a novel yeast strain *Candida stellimalicola* 4-ASe will enable to broaden the range of fodder commodities launched onto the market. Their applications relying on the analyses of biochemical blood tests, examination of intestinal microbiota of laying hens, efficiency of selenium incorporation in eggs, will promote enhanced yields, quality and profitability of poultry products, fabrication of foodstuffs preventing diseases provoked by selenium deficiency.

**References:**

1. Esmaeili S, Khosravi-Darani K. Selenium-Enriched Yeast: As Selenium Source for Nutritional Purpose. *Curr Nutr Food Sci* 2014;10(1):49-56. doi: 10.2174/1573401310011403281157 53.
2. Frączek A, Pasternak K. Selenium in medicine and treatment. *J Elem* 2013;18(1);145-163. doi:10.5601/jelem.2013.18.1.13.
3. Kieliszek M, Błażejak S, Kurek E. Binding and conversion of selenium in *Candida utilis* ATCC 9950 yeasts in bioreactor culture. *Molecules* 2017;22(3):352. doi: 10.3390/molecules22030352.
4. Kieliszek M. Selenium-Fascinating Microelement, Properties and Sources in Food. *Molecules* 2019;24(7):1298. doi:10.3390/molecules24071298.
5. Suchý P, Strakov E, Herzig I. Selenium in poultry nutrition: a review. *Czech J. Anim. Sci* 2014;59(11):495-503. doi:10.17221/7730-CJAS.
6. Boostani A, Sadeghi AA, Mousavi SN, Chamani M, Kashan N. The Effects of Organic, Inorganic, and Nano-Selenium on Blood Attributes in Broiler Chickens Exposed to Oxidative Stress. *Acta sci. vet.* 2015;43(1):1264.
7. Moroz I, Pavlyuk A, Sapunova L. A yeast strain adapted to selenium as a promising source of selenium fodder additives. Symposium with international participation: Modern biotechnologies – solutions to the challenges of the contemporary world. Chișinau 2021, 20-21 mai (online):152. doi:10.52757/imb21.094.
8. Prytkov Yu, Kistina A, Kiseleva K, Simonov G. Selenium in the rations of laying hens of *Lohmann Brown* cross-line. Composite feeds 2019;6:50-51. doi: 10.25741/2413-287X-2019-06-3-073. (in Russian)
9. Dorozkina EI, Kistina AA, Kukolina NV, Prytkov YuN. Application of organic selenium compound in the rations of laying hens of *Lohmann-Broun* cross-line [e-resource]. Ogarev-online 2017;1. Access mode: https://journal.mrsu.ru/arts/primenenie-organicheskogo-selena-v-racionax-kur-nesushek-krossa-lomann-braun. Access date: 13.09.2022. (in Russian)
10. Heindl J, Ledvinka Z, Englmaierová M, Zita L, Tůmová E. The effect of dietary selenium sources and levels on performance, selenium content in muscle and glutathione peroxidase activity in broiler chickens. *Czech J Anim Sci* 2010;55(12):572-578. doi:10.17221/2487-CJAS.
11. Rajashree K, Muthukuma T, Karthikeyan N. Comparative study of the effects of organic selenium on hen performance and productivity of broiler breeders. *Br Poult Sci* 2014;55(3):367-74. doi:10.1080/00071668.2014.921663.
12. Madkour M, Ali HM, Yassein SA, et al. Effect of dietary organic selenium supplement on growth and reproductive performance of japanese quail breeders and their progeny and its relation to antioxidation and thyroid activity. *Int J Poult Sci* 2015;14(6):317–324. doi:10.3923/ijps.2015.317.324.
13. Zia MW, Khalique A, Naveed S, Hussain J. Impact of selenium supplementation on productive performance and egg selenium status in native Aseel chicken. *Ital J Food Sci* 2016;15(4):649-657. doi:10.1080/1828051X.2016.1222247.
14. Liu H, Yu Q, Fang C, et al. Effect of selenium source and level on performance, egg quality, egg selenium content, and serum biochemical parameters in laying hens. *Foods* 2020;9(1): 68. doi:10.3390/foods9010068.

# THE ROLE OF PROBIOTIC LAB IN FEEDSTUFF

Sargsyan A.[2], Tkhruni F.[1], Agabekyan L.[2], Sargsyan M[2], Israyelyan A.[2]
[1]*Probiotics Production Sector, Scientific and Production Center "Armbiotechnology" NSPO, National Academy of Science, Armenia*
[2]*Laboratory of Microbiology, Artsakh Scientific Center State Non-Commercial Organization (SNCO), Armenia*
e-mail: anyuta.sargsyan@mail.ru

CZU:636.084.4+579.64 https://doi.org/10.52757/imb22.31

**Keywords:** cow, probiotic, LAB, feedstuff, protein nutritional value.

Frequent uncontrolled use of feed antibiotics in intensive production of feedstuff has led to the formation of resistant strains of pathogenic bacteria. The use of feed antibiotics has resulted in increased productivity of farm animals due to the suppression of the pathogenic microflora of the digestive system. The situation is exacerbated by animal stresses due to poor feed quality and poor conditions. Often there are cases of dysbacteriosis, especially in young animals, reduced cows' reproduction, excess live weight of young animals, increased infectious and alimentary (caused by defective unbalanced feeding) origin is growing diseases.

Based on the results of scientific of previous grants supported by the State Committee on Science of the Republic of Armenia and the ANSEF at the laboratory of Artsakh Scientific Center was developed a new technology for enrichment of feedstuff in accordance with the main nutritional resources of Artsakh and with probiotic lactic acid bacteria *Enterococcus durans* KE5, *Lactobacillus acidophilus* 1991, *Streptococus lactis* and *Streptococcus termophilus*.

The data show that after the use of probiotic bacteria, the quality of the feed increases. The activity of probiotic bacteria suppresses the growth of fungi and pathogenic microflora.

The results of the content of amino acids and protein during silage of the green mass of corn are given in Table 1.

**Table 1. The content of amino acids and protein source-grass from the Herher region**

| Source-corn from the Herher region | Amino acids, mg / ml | | | | | | | | | | | Protein, % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lys | Arg | Ala | Glut | Val | Iso | Tre | Met | Fal | Start | The amount of amino acids mg / ml | |
| Control | 1,6 | 1,2 | 1,6 | 2,4 | 2,4 | 2,4 | 0,8 | 3,2 | 0,4 | 0,8 | 16,8 | 16,5 |
| Consortium LAB | 1,6 | 0,8 | 1,2 | 0,4 | 1,6 | 2,4 | 1,2 | 1,6 | 0,4 | 0,8 | 12,0 | 28,0 |
| Consortium LAB+yeast | 1,6 | 1,2 | 1,2 | 0,8 | 1,6 | 2,4 | 1,2 | 1,6 | 0,4 | 0,8 | 12,8 | 24,0 |

The data obtained show that during silage of the green mass of corn, the addition of a consortium consisting of yeast and LAB or only LAB can increase the protein content by an average of 50%, which is higher than when silaging a mixture of grass, regardless of the source of its use.

**Conclusions**

The property of lactic acid bacteria to synthesize lactic acid is used for silage of green biomass. However, we have shown that the use of a consortium of LAB strains and yeast with probiotic properties leads to an increase in the content of protein and essential amino acids in silage, a decrease in its infectivity, and the effectiveness of the method used depends on the source and nature of the used method of green biomass and strains. Therefore, the use of starter culture from the consortium of investigated probiotics LAB and yeast in silage should be introduced as widely as possible, as they have a positive effect on the health of animals.

*Acknowledgments. This work was supported in the frame of the research projects MESC AR scs 19AA-002, ANSEF biotech 52-52.*

**SOME ASPECTS OF DEVELOPING MICROBIAL PREPARATIONS FOR PLANT PROTECTION**

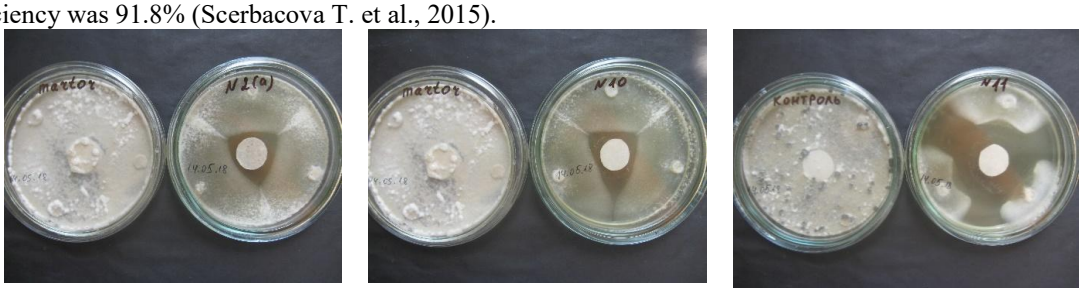
Scerbacova T.
*Institute of Genetics, Physiology and Plant Protection, Republic of Moldova*
e-mail: tatiana.scerbacova@igfpp.md

CZU:573.6.086:632.937 https://doi.org/10.52757/imb22.32

The basis of microbial means of plant protection against diseases is live cultures of microorganisms with high virulence and their metabolic products. The leading role in the biological control of plant diseases is assigned to microscopic fungi. A special place is occupied by the genus *Trichoderma* Pers. ex Fr. The advantages are a high growth rate, a wide range of antifungal activity, and simple equipment for cultivation on an industrial scale.

The biopreparation production technology constitutes the cultivation of the fungus-producer in a liquid nutrient medium in a bioreactor or on a microbiological shaker for 72-96 hours. An important step in obtaining effective biopreparations is the selection of the optimal nutrient medium for cultivating the bioagent. Modification of nutrient media according to the main sources of nutrition of microorganisms (carbon, nitrogen) promotes the formation of biologically active substances that have an inhibitory effect on phytopathogens. This action can be strengthened or weakened.

During the evaluation of the fungicidal action spectrum of the liquid biopreparation Gliocladin-SC (the active substance is the fungus *Trichoderma virens* Miller, Giddens, and Foster), 18 pathogenic agents of crop diseases causative agents were identified (Scerbacova T., 2019). Several liquid nutrient media were used in the present work. When the medium composition changed according to the carbon source, in addition to chlamydospores, conidia and blastospores were formed. The zones of *Sclerotinia sclerotiorum* pathogens inhibition growth (Fig. 1) and *Botrytis cinerea* expanded, and the antifungal effect against pathogens of fruit crops *Monilia cinerea* and *M. fructigena* also increased. The preparation fabricated on the base of that nutrient medium was tested on "Krupnoplodnyi" sweet cherries variety to suppress the development of moniliosis. After two treatments with 1% concentration, the disease development reduction efficiency was 91.8% (Scerbacova T. et al., 2015).




Media 2(a) Media 10 Media 11

**Figure 1.** Growth inhibition zones of the pathogen *S. sclerotiorum* with Gliocladin-SC biopreparation based on media with different compositions

In the result of the conducted research, it was found that for the successful application of Gliocladin-SC biopreparation in plant protection against a wide range of diseases, separate balanced nutrient media for controlling different groups of pathogens are needed.

*Acknowledgments. This research was carried out within the State Program project nr. 20.80009.7007.16. "Synergism between natural factors and ecologically harmless microbiological means of regulating the population density of harmful organisms for the protection of agricultural crops in conventional and ecological agriculture", funded by the National Agency for Research and Development of Republic of Moldova.*

**SURFACTANT-FORMING ACTIVITY OF BACTERIA *RHODOCOCCUS* SP. G13**

Shavela Y., Hubchyk K., Hlushen A.
*Institute of Microbiology NAS Belarus, Minsk, Belarus*
e-mail: shavela97@mail.ru

CZU:579.22 https://doi.org/10.52757/imb22.33

Bacteria of the genus *Rhodococcus* are widely used in the field of modern environmental biotechnology (bioremediation, utilization of toxic waste) due to their prevalent presence in anthropogenic disturbed ecotopes and availability of adaptive survival mechanisms in unfavorable environmental conditions [1]. Actinobacteria are characterized with the ability to synthesize bacterial cell components that ensure the neutralization of a wide range of xenobiotics in the process of recovery of contaminated ecosystems, due to the formation of gaseous and liquid n-alkanes [2]. One of the mechanisms for increasing the bioavailability of complex organic compounds for microbial cells is the synthesis of biosurfactants that reduce surface and interfacial tension and ensure the emulsification of hydrophobic substrates for their more efficient biodegradation [3].

In this research we investigated surfactant-forming and emulsifying activity of the oil-destroying bacterial strain *Rhodococcus* sp. G13 when cultivated on mineral medium containing various sources of nitrogen ($NaNO_3$, $KNO_3$, $(NH_4)_2SO_4$, $NH_4NO_3$) and phosphorus ($KH_2PO_4$, $K_2HPO_4$, $Na_2HPO_4$) in the presence of several carbon sources (glucose, ethanol, hexadecane). The emulsification index of hexadecane by *Rhodococcus* sp. G13 cells ranged from 23.8±1.5% to 54.1±1.2% depending on the salt composition of the mineral medium and the type of organic compounds as the carbon source are used. At the same time, when cell-free supernatant was used in the experiment, this index was about 2.76±0.16%. The ability of the bacterial cells to reduce the surface tension of the liquid was also evaluated, which was from 13.95±0.8% to 56.04±1.1% compared to the control medium. The colorimetric method [4] demonstrated that the level of biosurfactant synthesis by *Rhodococcus* sp. G13 reached up to 13.54±1.4 mg/L.

Research results indicate that surfactant synthesis, hydrophobic substrate solubilization and surface tension reduction occur most effectively under nitrogen-deficient cell culture conditions and using hexadecane as the sole carbon source. The obtained data also indicate that biosurfactants are located in the bacterial cell structure of *Rhodococcus* sp. G13.

**References**

1. Stress response in *Rhodococcus* strains / M. Pátek [et al.] // Biotechnology Advances. – 2021. – T. 53. – P. 107698.

2. Comparative genomics of the *Rhodococcus* genus shows wide distribution of biodegradation traits / D. Garrido-Sanz [et al.] // Microorganisms. – 2020. – T. 8. – №. 5. – P. 774.

3. Biotechnology of *Rhodococcus* for the production of valuable compounds / M. Cappelletti [et al.] // Applied Microbiology and Biotechnology. – 2020. – T. 104. – № 20. – P. 8567–8594.

6. Improving the bioremediation technology of contaminated wastewater using biosurfactants produced by novel bacillus isolates / O. M. Darwesh et al. // Heliyon. – 2021. – T. 7. – №. 12. – P. e08616.

*The work was performed within the task B21UZBG-010 "Obtaining industrial strains of microorganisms - superproducers of surfactants in order to create new biopreparations for environmental purification", sponsored by the Belarusian Republican Foundation for Basic Research.*

**ENTOMOPATHOGENIC BIOPESTICIDES - AN ALTERNATIVE INCREASING THE ADAPTABILITY OF PLANTS TO STRESS IMPACT AND ECO-FRIENDLY SOURCE FOR THE CONTROL OF PESTS**

Stingaci A., Serbacova T, Samoilova A., Zavtoni P., David T., Lungu A., Curiev L.
*Institute of Genetics, Physiology and Plant Protection, Republic of Moldova*
email: aurelia.stingaci@gmail.com

CZU:632.937 https://doi.org/10.52757/imb22.34

The use of chemical fertilizers and chemical plant protection products in unacceptable amounts has led to a steady decline in soil and crop productivity worldwide. Pesticides contribute to the pollution of ground and surface waters, and there are strong indications that long term exposure to POPs can have a negative impact on biodiversity (Topping et. al., 2020).

The current level of plant protection does not solve all problems of crop production, since pesticides are not able to protect plants from abiotic stresses. Meanwhile, losses from stress factors are estimated at 51-82% (Monastyrsky O.G., 2011), which significantly exceeds the losses from diseases and pests.

Biotic stress is any stress caused by living organisms like insects, viruses, bacteria, fungi, and arachnids. The plant never develops an adaptive immunity against biotic stress even on repeated exposures. For this very reason biotic stress is the major factor of pre-and post-harvest losses (Singla and Krattinger, 2016). Abiotic stress includes conditions such as drought, temperature fluctuations, high soil salinity, metal toxicity, and oxidative stresses. These stresses can cause permanent damage to a plant such as stunted growth, hampered metabolism, reduced yield, and change in genetic behaviour, leading to mutations in the progeny (Zaidi et al., 2014, Bhat et al., 2020).

The effects of drought and the losses incurred are long term. Groundwater pumping costs continue to rise (Lund et al., 2018), rainfall decreases every year, affecting non-irrigated agricultural land, inter-seasonal droughts and moisture deficits occur, resulting in inevitable drought-like conditions (Kaushik, 2015).

The aim of the work was to establish the possibility for application of entomopathogenic strains *Bacillus thuringiensis* ssp. *kurstaki* (Bt) and *Bacillus thuringiensis* ssp. *thuringiensis* (BT) in a tank mixture for spraying. For this purpose, the effect of the recommended and half concentrations of para-aminobenzoic acid (PABA) on the above-mentioned bacteria colonies was examined *in vitro*. Bacteria were cultivated in liquid mineral nutrient medium for 48 hours at 29°C to the titer of $10^9$ CFU/ml. The suspension was inoculated on the solid CGA nutrient media in Petri dishes. After bacterial cultures had grown for 24 hours, sterile disks (five disks per three Petri dishes) impregnated with biologically active substance emulsions were placed on their surfaces. After a week of incubation, the interaction of the studied concentrations of para-aminobenzoic acid (PABA) with bacterial culture was recorded. Bacterial growth inhibition zones were not found. This allows to assume that it is possible to combine working solutions of bioregulators with bacterial strains suspensions and at the same time to reduce the para-aminobenzoic acid (PABA) after effect. The similar results have been reported for *B. thuringiensis* mixtures with the pesticides Sumi-Alpha, Regent, Decis and *Pseudomonas* sp., *Bacillus sp.* with the pesticides Ridomil, Quadris, Raxil and Colfo-Super (Адрианов Ф.Д., 2011; Попов Ю.Б., 2008; Войтка Д.В.,2018).

*Acknowledgements. Research was carried out within the State Program project nr. 22.80013.5107.3BL "Methods of complex application of para-aminobenzoic acid derivatives and entomopathogenic microorganisms for the control of harmful organisms on fruit and vegetable crops", funded by the National Agency for Research and Development of Republic of Moldova.*

# THE POTENTIAL OF MAGNETITE-BASED NANOCOMPOSITES IN NANOPHYTOREMEDIATION OF SOILS POLLUTED BY POLYETHYLENE

Todiras V.[1], Prisacari S.[1], Corcimaru S.[1], Gutul T.[2]
[1]*Institute of Microbiology and Biotechnology, Republic of Moldova*
[2]*The "D. Ghitu" Institute of Electronic Engineering and Nanotechnologies, Republic of Moldova*
e-mail: vasiletodiras@gmail.com

CZU:579.64+631.4 https://doi.org/10.52757/imb22.35

The Republic of Moldova suffers from the problem of environmental pollution by plastics, including by the low-density polyethylene (LDPE). The accumulation of plastics by plants has negative consequences for the food security and sustainable development of the agriculture. It is suggested that over time soil pollution by plastics can threaten the successful functioning of the entire agricultural system.

The negative consequences of soil pollution by plastics impose the need of developing measures of remediation. Due to the lack of efficient chemical and physical methods for destroying plastics in soil, the attention has recently been directed towards developing biological degradation techniques, including the ones based on application of phytoremediation and nano-phytoremediation. However, the potential of these techniques in the cases of soil pollution by LDPE is understudied. The aim of this work was to estimate the potential of the magnetite-based nanocomposites in the nano-phytoremediation of soils contaminated by LDPE.

According to the obtained results, under the conditions of the vegetative experiments the LDPE treated by different magnetite-based nanocomposites and then introduced into a soil collected from the landfill near Slobozia-Dușca (contaminated with different pollutants including LDPE) did not have toxic effects on the development of soybean plants. More than that, the plants from the variant where the soil was treated with the LDPE covered by the $MgFe_2$/PVPmax nanocomposite and where the seeds were inoculated by a specific rhizobia strain had the highest dry mass that was statistically different from most variants: respectively, +44.4% and +19.4% as compared to the absolute and "inoculated" controls, and +38.0% as compared to the variant where the LDPE was without nanocomposites and the seeds – without inoculation. Also, the covering of LDPE by this nanocomposite significantly stimulated the root length (up to +62.2% comparing to the absolute control) and contributed to a 42.8% increase in the efficiency of seed inoculation by specific rhizobia (increased the mass of the root nodules). It was observed that the endosymbiosis with rhizobia was not possible without prior seed inoculation by a specific strain, implying that the soil was absolutely toxic to the aboriginal rhizobia.

**Conclusions:**

1. The magnetite-based nanocomposites were not toxic for the tested legume plants and for their endosymbiosis with the tested rhizobia strain.
2. The $MgFe2/PVP_{max}$ nanocomposite had a considerable potential in the matters of nanophytoremediation of the LDPE contaminated soils, manifested via significant stimulation of the growth of the legume plants and of their endosymbiosis with the specific rhizobia.

*The presented data were obtained within the research project "Microbial tools for degradation of non-recyclable plastics waste", registered under code 20.80009.7007.03 in the State Program for 2020-2023, funded by the National Agency for Scientific Research and Development of the Republic of Moldova.*

# GROWTH DYNAMICS OF NITROGEN-FIXING BACTERIA AT INCREASED SALINITY

Toplaghaltsyan A.[1], Karapetyan Zh.[1], Keleshyan S.[1], Avetisova G.[1,2],
Melkonyan L.[1,2], Tsarukyan G.[1], Ghochikyan V.[1]
[1] *Scientific and Production Center "Armbiotechnology" NAS RA,*
[2] *Yerevan State University*
e-mail: anna.toplaghaltsyan@gmail.com

CZU:579.64+631.461 https://doi.org/10.52757/imb22.36

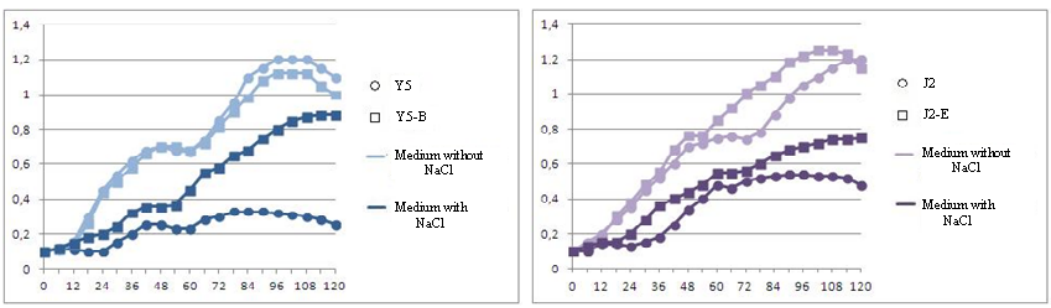
Nowadays, one of the main problems in the field of agriculture is the process of soil salinization. Salinity has already affected more than 7% of the Earth's land area. One of the options for combating salinity is to minimize the use of harmful chemicals. Instead, environmentally friendly means should be used to restore saline arable soils and increase their fertility [1]. One of the widely used means are biofertilizers based on nitrogen-fixing microorganisms. These biofertilizers help plants absorb nutrients in saline conditions, leading to increased plant tolerance to salinity. All this has a positive effect on the process of restoration of saline soils, as well as on the improving the quality and quantity of the crop [2].

The purpose of this work was studying the growth dynamics of nitrogen-fixing strains in the presence of NaCl. The objects of research were cultures Y5 and J2, previously isolated by us from saline soils of the Armavir region of Armenia, and osmo-resistant mutants Y5-B and J2-E obtained on their basis.

Cultures were grown for 5 days in Burk's broth medium on an Innova 43 shaker (30 °C, 220 rpm) in the presence of 1.7% NaCl. A salt-free medium was used as a control. Sampling was carried out every 12 hours and the optical density was measured at a wavelength of 600 nm.

The difference in the dynamics of cultures growth in saline and non-saline conditions is shown in Figure 1. The dynamics of the growth of strain Y5 in a salt-free medium was similar to its mutant Y5-B, but in the presence of NaCl it showed a small growth, in contrast to its mutant capable of growing in a salt medium. In the case of J2, on a salt-free medium the strain showed more passive growth compared to its J2-E mutant. This pattern is also present in the nutrient medium containing NaCl.

As a result, it can be concluded that osmotic-resistant mutants of strains Y5 and J2 are able to maintain their normal vital activity in a saline environment, in contrast to the original cultures.




**Figurre 1.** Growth dynamics of nitrogen-fixing strains

## References

1. Mohan V., Menon S. (2015) Diversity Status of Beneficial Microflora in Saline Soils of Tamil Nadu and Pudhucherry in Southern India. *J Acad Ind Res*, 3(8), pp. 384-392.
2. Mokula R., Charyulu P. (2012) Nitrogen fixation by the native *Azospirillum* spp. isolated from rhizosphere and non-rhizosphere of foxtail millet. *Asian J Biol Life Sci*, 1(3), pp. 213-218. https://www.ajbls.com/article/2012/1/3/213-218

## ENZYMATIC ACTIVITY OF NITROGEN-FIXING SOIL BACTERIA

Toplaghaltsyan A.[1], Karapetyan Zh.[1], Keleshyan S.[1], Avetisova G.[1,2],
Melkonyan L.[1,2], Tsarukyan G.[1], Ghochikyan V.[1]
*1 Scientific and Production Center "Armbiotechnology" NAS RA, Armenia*
*2 Yerevan State University, Armenia*
e-mail: anna.toplaghaltsyan@gmail.com

CZU:631.461.5+579.2 https://doi.org/10.52757/imb22.37

Soil is a medium for more than 100 enzymes. During soil deterioration the change of enzymes occurs much sooner than of other parameters in the soil so they are considered the best indicators of soil health. These enzymes play a vital role in supporting soil ecology and health by direct agents of the biological catabolism of soil organic and mineral components. Enzymatic activities in the soil are mainly of microbial origin. In a number of potential bacterial enzymes that play an important role in maintaining soil health, some of the important ones are protease, lipase, cellulase, amylase and urease [1].

The aim of this work was the study of enzymatic activity in nitrogen-fixing bacteria, such as protease, lipase, cellulose, amylase and urease. The bacteria used in the experiments were *Agrobacterium* sp. strain M-1 (MN717167) and *Agrobacterium* sp. strain Y-2 (MN721294), previously isolated by us from saline soils of the villages Mrgashat and Yeghegnut of Armenia, respectively [2].

Proteolytic activity of nitrogen-fixing strains was determined using Skim Milk Agar, lipolytic activity - by Burk's Agar with 2% Tween 80, cellulolytic activity - by Cellulose Congo Red Agar. Bacterial suspension ($10^{7}$-$10^{8}$ CFU/ml) was added into punch holes in the agar and plates were incubated at 30 °C during 3-7 days. Enzymatic activity was detected by clear zones around holes.

In case of amylolytic activity Starch Agar was used. A fresh colony of bacteria was streaked on the surface of the agar by double streak and was incubated for 3-5 days at 30 °C. Then the surface of the agar was flooded with Gram's iodine solution. A clear zone surrounding the bacterial growth confirmed the hydrolysis of starch.

Urease activity was investigated by using Urea broth. The cultures were inoculated separately into test tubes and incubated at 30 °C for 4 days. The appearance of a deep pink color indicated a positive result.

The results of the experiments presented in Table 1 show the presence of a fairly wide range of enzymes in cultures *Agrobacterium* sp. strain M-1 and *Agrobacterium* sp. strain Y-2.

**Table 1. Enzymatic activity of nitrogen-fixing bacteria**

| Nitrogen-fixing bacteria | Enzymatic activity | | | | |
|---|---|---|---|---|---|
| | Protease | Lipase | Cellulase | Amylase | Urease |
| *Agrobacterium* sp. strain M-1 | - | + | + | + | + |
| *Agrobacterium* sp. strain Y-2 | + | + | + | + | + |

Therefore, it can be assumed that the use of *Agrobacterium* sp. strain M-1 and *Agrobacterium* sp. strain Y-2 with a variety of enzymatic activities can provide the soil with the enzymes necessary for the normal course of global carbon and nutrient cycles.

### References

1. Das S.K., Varma A. (2010) Role of Enzymes in Maintaining Soil Health. *Soil Enzymology*, 22, pp. 25-42. https://doi.org/10.1007/978-3-642-14225-3_2
2. Keleshyan S.K., Karapetyan Z.V., Toplaghaltsyan A.G. et al. (2022) Obtaining Osmo-resistant Mutants in Nitrogen-Fixing Bacteria Isolated from Saline Soils. *Curr Microbiol,* 79, 251. https://doi.org/10.1007/s00284-022-02948-9.

# ENZYMATIC ACTIVITY OF NITROGEN-FIXING BACTERIA ISOLATED FROM ARMENIAN SALINE SOILS

Toplaghaltsyan A.,[1] Karapetyan Zh.,[1] Keleshyan S.,[1] Avetisova G.,[1,2] Melkonyan L.,[1,2] Tsarukyan G.,[1] Ghochikyan V.[1]

*1 Scientific and Production Center "Armbiotechnology" NAS RA, Armenia*

*2 Yerevan State University, Armenia*

e-mail: anna.toplaghaltsyan@gmail.com

CZU:579.26:631.46 https://doi.org/10.52757/imb22.38

**Summary**

Nitrogen-fixing bacteria with phytostimulating potential have great interest in the modern world. The aim of this work was to study the enzymatic activity of nitrogen-fixing strains *Agrobacterium* sp. M-1 and *Agrobacterium* sp. Y-2. Studies have shown that these strains are capable to produce proteases, lipases, cellulases, amylases and ureases, enzymes that play a vital role in maintaining soil health. In addition, these bacteria have several significant characteristics: salt tolerance, pH stability, maintaining of viability at low and high temperatures. Therefore, the above strains have every chance to become the basis for the creation of a multifunctional biofertilizer in the future.

**Keywords:** soil enzymes, biological catabolism, microbes, soil health, nirogen-fixing bacteria

## Introduction

Soil is a medium for more than 100 enzymes that play a fundamental role in supporting soil ecology and health. They are direct agents of the biological catabolism of the organic and mineral components of the soil and with their help the decomposition of organic residues and the cycle of nutrients occur in the nature. During soil deterioration the change of enzymes occurs much sooner than of other parameters in the soil. That's why they are the best indicators of soil health and by evaluation of their activities it is possible to determine whether soil processes are proceeding satisfactorily [1].

In a number of potential bacterial enzymes that play an important role in maintaining soil health and in the transformation of different nutrients for plants, some of the important ones are protease, lipase, cellulase, amylase and urease. High productivity of crops is associated with the activity of these enzymes, since they convert plant and animal waste into humus, which is then completely decomposed into free nutrients consumed by plants [2].

Proteases are degradative enzymes, catalyzes proteolysis - breaking down of proteins into smaller polypeptides or single amino acids. They are present in all forms of life, such as animals, plants, and microbes. Proteases are regulators of physiological processes, controlling the activation, synthesis and turnover of proteins. Microbial proteases are the most used enzymes worldwide and account for two thirds of commercial proteases. They are preferred over plant and animal proteases due to having all the characteristics required for industrial applications: high productivity, less consumption of time, less requirement of space, high genetic manipulation and cost effectiveness [3-4].

Lipases are family of versatile enzymes that catalyze triglycerides into free fatty acids and glycerol. They can be obtained from several sources: animal, plants, and microbes. Lipase has a wide spectrum of activity and is involved in many reactions [5]. Lipase activity is one of the primary values for all living organisms, since it balances physiological processes of digestion and absorption, as well as the metabolism of fats and lipoproteins. Thereby, lipases are multi-purpose biological catalysts and are used in several industries such as biodiesel, food, textiles, pharmaceuticals, medicine, etc., due to their substrate specificity and ability to catalyze reactions at extreme pH, temperature and presence of metal ions [6].

Cellulase is an enzyme from the class of hydrolases that breaks down the cellulose molecule into monosaccharides, shorter polysaccharides and oligosaccharides. Cellulose is a widespread biomass in the nature, since it is the main component of cell walls of plants. Abundant availability of cellulose makes it an attractive raw material for producing many industrially products, cause can be converted to glucose which is a multiutility product. It can be done by the cellulolysis - the process of cellulose hydrolysis, which is basically the biological process controlled by the enzymes of cellulase system [7]. Cellulase enzyme system comprises three classes of soluble extracellular enzymes and only the synergy of them makes the complete cellulose hydrolysis to glucose. Many microbes, mostly bacteria and fungi have a cellulosic activity, but potential of bacteria is better due to their high rate of growth [8].

Amylases are starch-degrading enzymes, which occupy a quarter of all enzymes used in the global industry. Amylases are found in microorganisms, plants and animals, but in industrial processes such as food and pharmaceutical industries have basically used microorganism amylases, mainly bacteria and fungi by their higher stability and easy manipulating. Some of the major industrial enzymes from group of amylases are α-amylases preferred due to their ability act at high temperatures and alkaline pH. Function of α-amylases is hydrolyzed of α-1,4-glycosidic linkages of the starch leading to the formation of dextrins. Starch is a complex carbohydrate that exists in many vegetables and fruits. Plants create these polymers for storing of glucose, creating during process of photosynthesis [9-10].

Urease is a widespread in nature nickel-containing metalloenzyme, catalyses the hydrolysis of urea to ammonia and carbamate, and thus generates the preferred nitrogen source of many organisms. Ureases are found in numerous bacteria, fungi, algae, plants, and some invertebrates. It is a predominant enzyme among the soil N cycle enzymes. Urease activity is a biological indicator of the soil. It allows to management the amount of urea fertilizer added to the soil, because as a result of the hydrolysis of urea-containing fertilizers by bacterial ureases, an increase in soil pH occurs. Subsequently, excessive addition of urea fertilizer to the soil can be prevented [11-13].

Some of the representatives of microbes with enzymatic activity are nitrogen-fixing bacteria, such as *Rhizobium*/*Agrobacterium* group, genera *Azotobacter* and *Pseudomonas*, etc. They have a good effect on the growth and development of the crops, leading to an increase in the yield. They enhance biological nitrogen fixation, help in the synthesis of biologically active substances, make certain nutrients available to plants such as C, N, P, and also protect plants from phytopathogens because they are good antagonists [19, 25, 27].

Nitrogen is one of the most abundant elements in surface and atmosphere of Earth; moreover, it's one of the essential macronutrients for plant growth. Unfortunately, molecular nitrogen is not available for uptake by plants. Plants absorb nitogen in the form of ammonium salts, which are formed in the soil due to nitrogen-fixing bacteria. The latter convert molecular nitrogen fixed from the atmosphere into ammonia, which transfomed into ammonium salts in the soil. That's why bacteria supporting nitrogen fixation and plant growth are undoubtedly the most the vital resources for increasing productivity in agriculture [14].

**The aim** of this work was the study of enzymatic activities of protease, lipase, cellulose, amylase and urease in nitrogen-fixing bacteria isolated from saline soils of Armenia.

**Materials and methods**

**Strains:** The bacteria used in the experiments were *Agrobacterium* sp. strain M-1 (Genbank accession number: MN721294) and *Agrobacterium* sp. strain Y-2 (Genbank accession number: MN717167), representatives of *Rhizobium*/*Agrobacterium* group, previously isolated by us from saline soils of the villages Mrgashat and Yeghegnut of Republic of Armenia, respectively [15].

**Growth medium:** Burk's Agar was used as a nutrient medium with the following composition: 20.0 g/l sucrose, 0.640 g/l K2HPO4, 0.160 g/l KH2PO4, 0.20 g/l MgSO4 7H2O, 0.20 g/l NaCl, 0.050 g/l CaSO4 2H2O, 0.50 g/l Na2MoO4 · 2H2O, 3 g/l FeSO4 7H2O, 15.0 g/l agar; pH 7.3 [16].

To determine the enzymatic activity of the studied nitrogen-fixing bacteria, special mediums were prepared: Skim Milk Agar for proteolytic activity, Burk's Agar with Tween 80 for lipolytic activity, Congo-Red Agar for cellulolytic activity, Starch Agar for amylolytic activity and Urea Broth for ureolytic activity.

Skim Milk Agar – 5.0 g/l pancreatic digest of casein, 2.50 g/l yeast extract, 1.0 g/l glucose, 70.0 g/l skimed milk powder, 15.0 g/l agar; pH 7.0 [17].

Burk's Agar with Tween 80 – composition of the Burk's medium with the addition of 2% Tween 80 (v/v); pH 7.3 [16].

Congo-Red Agar – $KH_2PO_4$ – 0.5, $MgSO_4$ – 0.25, cellulose – 2.0, Congo-Red – 0.2, gelatin – 2.0, agar – 15.0; pH 6.8–7.2 [8].

Starch Agar – 3.0 g/l beef extract, 10.0 g/l soluble starch, agar – 12.0; pH 7.5 [18].

Urea Broth – 9.50 g/l K2HPO4, 9.10 g/l KH2PO4, 20.0 g/l urea, 0.10 g/l yeast extract, 0.010 g/l phenol red; pH 6.8 [19].

**Determination of enzymatic activity:** In order to obtain fresh cultures for use in the experiments, the strains were each time pre-seeded on Burk's Agar and incubated for 2 days at 30 °C. Bacterial

suspensions were prepared in physiological saline solution (0.96%, w/v) with the $10^7$–$10^8$ CFU/ml end concentration.

*Proteolytic activity*: Protease production was determined using Skim Milk Agar medium. 0.2 ml of bacterial suspension was added into punch holes (5 mm diameter) in the agar and plates were incubated at 30 °C during 3 days. Formation of clear zone around the holes was considered as availability of proteolitic activity [17].

*Lipolytic activity*: Presence of lipase enzymes was determined by Burk's medium with Tween. Bacterial suspension was added into punch holes and Petri dishes were incubated at 30 °C during 5 days. The presence of a clear zone around the bacterial inoculum was indicated as the degradation of the Tween [20].

*Cellulolytic activity*: Cellulose degradation was study using Congo-Red Agar. Suspension was transferred into holes and was growth for 7 days at 30 °C. Manifestation of Congo red discoloration zone was taken as positive result [7].

Above three activities were measured by proteolytic index (PI), lipolytic index (LI) and cellulolytic index (CI):

$$PI\ or\ LI\ or\ CI = \frac{diameter\ of\ zone - diameter\ of\ punch\ hole}{diameter\ of\ punch\ hole}.$$

*Amylolytic activity*: Presence of amylases was by Starch Agar medium. A fresh colony of bacteria was streaked on the surface of the agar and was incubated for 3-5 days at 30 °C. Then the surface of the agar was flooded with Gram's iodine solution (3 ml). A clear zone surrounding the bacterial growth confirmed the hydrolysis of starch [21].

*Ureolytic activity*: Activity of ureases was investigated by using Urea broth. 0.1 ml of bacterial suspension was added into test tubes with broth (5 ml) and incubated at 30 °C for 4 days. The appearance of a deep pink color indicated a positive result [19].

**Statistical analysis:** Data analysis was carried out ANOVA by Dunnett's test ($p<0.05$) using Minitab 17.1 statistical program. All the experiments were conducted at least for 3 times in triplicates.

**Results and discussions**

The role of nitrogen-fixing bacteria in agriculture is of great interest due to their phytostimulating potential, bioregulatory properties and large number in the rhizosphere. Moreover, their enzymatic activities are also important for soil health and ecology, as evidenced by various literary data [20-22].

Enzymes are direct agents of the biological catabolism of the organic and mineral components of the soil. High productivity of crops is associated with the activity of these enzymes, since they convert plant and animal waste into free nutrients consumed by plants. Enzymes are present in all forms of life, such as animals, plants, microorganisms, etc. [13]. In worldwid the most used are microbial enzymes which are preferred due to their high productivity, less consumption of time and space, high genetic manipulation and cost effectiveness. In a number of potential bacterial enzymes that play an important role in soil health and in the transformation of different nutrients for plants, some of the importants are protease, lipase, cellulase, amylase and urease: enzymes that hydrolyze proteins, fatty acids, cellulose, starch and urea, respectively [2]. In addition, it is known that secretion of lytic enzymes (lipase, cellulase and protease) by microbes can contribute the inhibition of plant pathogens' growth [20].

In order for such potential bacteria in the composition of biofertilizers to be useful for plants, they must at first successfully take root in the soil, and then be able not only to survive, but also to maintain their vital activity, which is not always possible due to various harsh environmental conditions. That's why, in this work were study the nitrogen-fixing bacteria *Agrobacterium* sp. strain M-1 and *Agrobacterium* sp. strain Y-2 with Genbank accession numbers MN721294 and MN717167 respectively, which were previously isolated by us from saline lands of Armenia (with 2.1% average salt value, w/v) [22]. In addition of salt tolerance, these bacteria are also resistant to various pH values (in the range from 6 to 10) and are able to maintain their viability in conditions from low to high temperatures (in the range from -20 °C to +55 °C) [15].

Screening media Skim Milk Agar, Burk's Agar with Tween 80, and Congo-Red Agar were used to determine proteolytic, lipolytic, and cellulolytic activity, respectively. With a positive result, a clear zone was formed around the hole punch, the diameter of which was measured and, on its basis, the enzyme index

was determined. The results of enzymatic activities study indicated that both strain of *Agrobacterium* (M-1 and Y-2) were positive in the lipase test and cellulase test, but protease test was shown positive result only in strain Y-2 (Table 1).

**Table 1. Proteolytic, lipolytic and cellulolytic activities of nitrogen-fixing bacteria**

| № | Nitrogen-fixing bacteria | Enzymatic activity | | | | | |
|---|---|---|---|---|---|---|---|
| | | Protease | | Lipase | | Cellulase | |
| | | Zone (mm) | PI | Zone (mm) | LI | Zone (mm) | CI |
| 1 | *Agrobacterium* sp. strain M-1 | 60 | 5.0 | 65 | 5.5 | 52 | 4.2 |
| 2 | *Agrobacterium* sp. strain Y-2 | 58 | 4.8 | 42 | 3.2 | 45 | 3.5 |

There are many data in the literature about the enzymatic activities of nitrogen-fixing bacteria. In one were shown that almost all study *Pseudomonas* strains have protease activity, in contrast to lipases and cellulases, which were found only in *P. chlororaphis* TSAU13 [20]. A similar result was observed by Jha et al. where fluorescent strains of *Pseudomonas* sp. were shown proteolytic activity, but only one of them has cellulases [17]. Among the representatives of *Rhizobium*, the presence of proteolytic activity is most common, for example proteases were finding in nine isolates of *Rhizobium* sp. from Indonesian soils [23] as well as in *Rhizobium* sp. strain R-986 and *Bradyrhizobium* sp. strain R-993 isolated from the soils of the Central Amazonian floodplain [24]. In addition, De Oliveira et al. discovered that maximal protease activities were exhibited when the cell growth reached the stationary phase [24]. In case of genus *Azotobacter* there are many studyes showing absence of cellulolytic activities and presence of protease activity [25-26]. Alsalim showed *Azotobacter chroococcum*'s ability to produce proteases and lipases [27].

In the case of determination of amylolytic activity, after incubation, the surface of the starch agar was covered with a solution of Gram's iodine, which reacts with starch and stains the agar in a dark blue color. The appearance of a light zone around the bacterial growth indicates the hydrolysis of starch by the bacteria due to the production of amylases [28]. Results were shown that both *Agrobacterium* sp. strain M-1 and *Agrobacterium* sp. strain Y-2 have amylolytic activity (Fig. 1a).

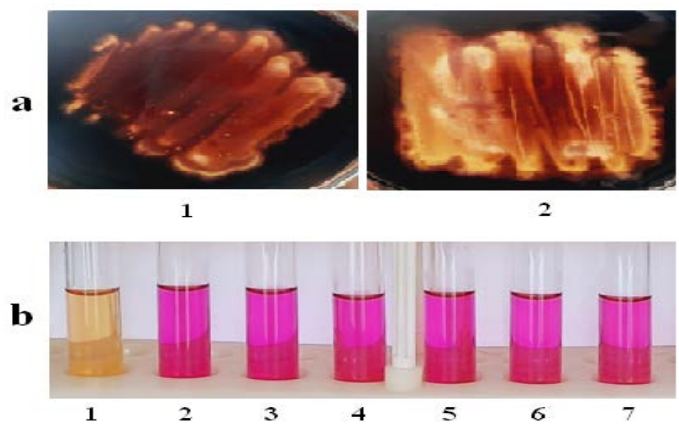



**Figure 1.** Amylolytic and ureolytic activities of of nitrogen-fixing bacteria

**a)** amylolitic activity - *Agrobacterium* sp. strain M-1 (a1) and *Agrobacterium* sp. strain Y-2 (a2) with positive result: the appearance of a light zone around the bacterial growth indicates the hydrolysis of starch

**b)** ureolytic activitiy – Control of Urea Broth (b1), *Agrobacterium* sp. strain M-1 (b2-b4) and *Agrobacterium* sp. strain Y-2 (b5-b7) with positive result: the pink color will develop due to the hydrolysis of urea and the formation of ammonia, leading to a change in the pH of the medium from neutral to alkaline

Study of ureolytic activity was done by Urea broth medium. Positive result was considered when the medium went from light orange to pink. The pink color was formed due to a change in pH from neutral to alkaline, which occurs due to the formation of ammonia in the medium in consequence to the hydrolysis

of urea by the urease enzymes [29]. The study of ureolytic activity showed that in all the studied samples the color of the medium became pink, i.e. they have a positive result (Fig. 1b).

There are data in the literature about starch hydrolysis by different nitorgen-fixing bacteria, e.g., by *Azotobacter chroococcum* [30] and by all studied isolates of *Pseudomonas* except Rh01 and Rh3 by Jha et al. [17], but *Rhizobium* isolates, located in symbiotic relationship with Groundnut, gave negative results for starch hydrolysis [21]. In case of urease activity positive result is meet in upland cotton-associated bacterial strains *Azotobacter chroococcum* AC1 and AC10 [25] and in all isolates of Jha et al. [17].

As seen from the literature data, the picture of enzymatic activities is very diverse and depends from various factors, for example the genus and species of bacteria, the environment conditions and so on.

Based on the research findings, it can be assumed that the use of *Agrobacterium* sp. strain M-1 and *Agrobacterium* sp. strain Y-2 with a variety of enzymatic activities can provide the soil with the enzymes necessary for the normal course of global carbon and nutrient cycles.

**Conclusions**

The work resulted in the detection of the variety of enzymatic activities in bacteria Agrobacterium sp. strain M-1 and Agrobacterium sp. strain Y-2. In addition, previous studies have shown their salt tolerance (under until 2.1% salts existence in environment), pH stability (in the range 6-10) and maintain of their viability in low to high temperatures (in the range from -20 °C to +55 °C). Based on these positive qualities, Agrobacterium sp. strain M-1 and Agrobacterium sp. strain Y-2 strain have every prospect of becoming the basis for the creation of a multifunctional biofertilizer.

**References:**

1. Das SK, Varma A. *Soil Enzymology: Role of Enzymes in Maintaining Soil Health.* Heidelberg, Germany: Springer Berlin; 2010. https://doi.org/10.1007/978-3-642-14225-3_2.
2. De Souza Silva CM, Fay EF. *Soil Health and Land Use Management: Effect of Salinity on Soil Microorganisms.* London, United Kingdom: IntechOpen; 2012. https://doi.org/10.5772/2516.
3. Hamza TA. Bacterial protease enzyme: Safe and good alternative for industrial and commercial use. Int. j. chem. biochem. 2017; 3(1): 1-10.
4. Razzaq A, Shamsi S, Ali A, Ali Q, Sajjad M, Malik A, Ashraf M. Microbial proteases applications. Front. Bioeng. Biotechnol. 2019; 7: 110. https://doi.org/10.3389/fbioe.2019.00110.
5. Chandra P, Enespa, Singh R, Arora PK. Microbial lipases and their industrial applications: A comprehensive review. Microb. Cell Fact. 2020; 19(1): 169. https://doi.org/10.1186/s12934-020-01428-8.
6. Yao W, Liu K, Liu H, Jiang Y, Wang R, Wang W, Wang T. A valuable product of microbial cell factories: Microbial lipase. Front. Microbiol. 2021; 12: 743377. https://doi.org/10.3389/fmicb.2021.743377.
7. GRATA K. Determining cellulolytic activity of microorganisms. Chem. Didact. Ecol. Metrol. 2020; 25(1-2): 133-143. https://doi.org/10.2478/cdem-2020-0010.
8. Sethi S, Datta A, Gupta BL, Gupta S. Optimization of cellulase production from bacteria isolated from soil. ISRN Biotechnol. 2013; 2013: 985685. http://dx.doi.org/10.5402/2013/985685.
9. Mehta D, Satyanarayana T. Bacterial and archaeal α-amylases: Diversity and amelioration of the desirable characteristics for industrial applications. Front. Microbiol. 2016; 7:1129. https://doi.org/10.3389/fmicb.2016.01129.
10. De Souza PM, De Oliveira Magalhães P. Application of microbial alphaamylase in industry – a review. Braz. J. Microbiol. 2010; 41: 850-861.
11. Krajewska B, Van Eldik R, Brindell M. Temperature- and pressure-dependent stopped-flow kinetic studies of jack bean urease. Implications for the catalytic mechanism. J. Biol. Inorg. Chem. 2012; 17(7): 1123-1134. https://doi.org/10.1007/s00775-012-0926-8.
12. Glibert P, Harrison J, Heil C, Seitzinger S. Escalating worldwide use of urea – a global change contributing to coastal eutrophication. Biogeochemistry. 2006; 77(3): 441–463. https://doi.org:10.1007/s10533-005-3070-5.
13. Inamdar A., Sangawe V., Adhapure N. *Rhizosphere Engineering: Enzymes in rhizosphere engineering.* Cambridge, USA: Academic Press; 2022. https://doi.org/10.1016/C2020-0-03385-2.
14. Vats S., Srivastava P., Saxena S., Mudgil B., Kumar N. *Soil Nitrogen Ecology: Beneficial effects of nitrogen-fixing bacteria for agriculture of the future.* Cham, Switzerland: Springer Cham; 2021. https://doi.org/10.1007/978-3-030-71206-8.
15. Keleshyan SK, Karapetyan ZV, Toplaghaltsyan AG, Avetisova GY, Melkonyan LH, Vardanyan AA, Ghochikyan VT. Obtaining osmo-resistant mutants in nitrogen-fixing bacteria isolated from saline soils. Curr. Microbiol. 2022; 79: 251. https://doi.org/10.1007/s00284-022-02948-9.

16. Stella M, Suhaimi M. Selection of suitable growth medium for free-living diazotrophs isolated from compost. JTAFS. 2010; 38(2): 211– 219.
17. Jha BK, Pragash MG, Cletus J, Raman G, Sakthivel N. Simultaneous phosphate solubilization potential and antifungal activity of new fluorescent pseudomonad strains, Pseudomonas aeruginosa, P. plecoglossicida and P. mosselii. World J. Microbiol. Biotechnol. 2009; 25: 573–581. https://doi.org/10.1007/s11274-008-9925-x.
18. Hemraj V, Diksha S, Avneet G. A review on commonly used biochemical test for bacteria. Innovare J. Life Sci. 2013; 1(1): 1–7. https://innovareacademics.in/journals/index.php/ijls/article/view/30
19. Patel MV, Patel RK. Indole-3-acetic acid (iaa) production by endophytic bacteria isolated from saline dessert, the little runn of kutch. CJM. 2014; 3(2): 17-28.
20. Egamberdieva D, Kucharova Z. Selection for root colonising bacteria stimulating wheat growth in saline soils. Biol. Fertil. Soils. 2009; 45: 563–571. https://doi.org/10.1007/s00374-009-0366-y
21. Hossain A, Gunri SK, Barman M, Sabagh AE, Teixeira Da Silva JA. Isolation, characterization and purification of Rhizobium strain to enrich the productivity of groundnut (Arachis hypogaea L.). Open Agric. 2019; 4: 400-409. https://doi.org/10.1515/opag-2019-0040.
22. Keleshyan S, Karapetyan Z, Toplaghaltsyan A, Avetisova G, Melkonyan L, Ghochikyan V. Characteristics of the microflora of saline soils of Armenia. SPFP. 2019; 1: 76-85.
23. Purwaningsih S, Agustiyani D, Antonius S. Diversity, activity, and effectiveness of Rhizobium bacteria as plant growth promoting rhizobacteria (PGPR) isolated from Dieng, central Java. IRAN.J. MICROBIOL. 2021; 13(1): 130-136. https://doi.org/10.18502/ijm.v13i1.5504.
24. De Oliveira AN, De Oliveira LA, Andrade JS. Production and some properties of crude alkaline proteases of indigenous Central Amazonian rhizobia strains. Braz. Arch. Biol. Tec hnol. 2010; 53(5): https://doi.org/10.1590/S1516-89132010000500024**.**
25. Romero-Perdomo F, Abril J, Camelo M, Moreno-Galván A, Pastrana I, Rojas-Tapias D, Bonilla R. Azotobacter chroococcum as a potentially useful bacterial biofertilizer for cotton (Gossypium hirsutum): Effect in reducing N fertilization. Rev. Argent. Microbiol. 2017; 49(4): 377-383. https://doi.org/10.1016/j.ram.2017.04.006.
26. Farajzadeh D, Yakhchali B, Aliasgharzad N, Bashir NS, Farajzadeh M. Plant growth promoting characterization of indigenous Azotobacteria isolated from soils in Iran. Curr. Microbiol. 2012; 64(4): 397-403. https://doi.org/10.1007/s00284-012-0083-x.
27. Alsalim HAA. Assessment of Azotobacter chroococcum's ability to produce hydrolytic enzymes and enhance growth of *Vicia faba*. Baghdad Sci. J. 2019; 16(1): 0034. https://doi.org/10.21123/bsj.2019.16.1.0034.
28. Madigan MT, Martinko JM, Stahl DA, Clark DP. *Brock biology of microorganisms.* San Francisco, USA: Benjamin Cummings; 2012.
29. Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, Woods G. *Koneman's color atlas and textbook of diagnostic microbiology.* Philadelphia, USA: Lippincott Williams and Wilkins; 2006.
30. Abdel-Hamid MS, Elbaz AF, Ragab AA, Hamza HA, Halafawy KA. Identification and characterization of *chroococcum* isolated from some egyptian soils. JACB.2010;1(2):93-104. https://doi.org/10.21608/JACB.2010.88790

# THE STUDY ON THE DYNAMICS OF THE ACCUMULATION OF SULFATED EXOPOLYSACCHARIDES IN THE CULTURAL LIQUID DURING THE CULTIVATION OF SPIRULINA IN THE PRESENCE OF THE COORDINATIVE COMPOUND [CUL(NO3)2]

Turcan O.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
Email: turcanolga2019@mail.ru

CZU:582.232:57.08 https://doi.org/10.52757/imb22.39

*Spirulina platensis* is one of the key organisms that has shown interesting results in the treatment of some tumors, viral infections and immunodeficiency. Spirulina polysaccharides possess antitumor, antioxidant, immunomodulatory and antiviral activity. Thus, the study of these bioactive substances is of great interest with a view to their application in various fields (pharmaceutics, food, bioremediation, etc.). In order to establish the cultivation period required for the maximum accumulation of exopolysaccharides (EPS), the dynamics of the accumulation of sulfated exopolysaccharides in spirulina was studied.

As a stimulator and regulator of EPS synthesis, the coordinative compound [$CuL(NO_3)_2$] was selected in a concentration of 2 mg/l, which had a maximum effect on their synthesis, when cultivating spirulina for 7 days. Thus, the cultivation of spirulina was carried out for 26 days, during which the content of sulfated exopolysaccharides was recorded on each day of cultivation.

At the end of the experiment, it was established that the accumulation of sulfated exopolysaccharides in the spirulina culture liquid occurs gradually, and the coordinating compound [CuL(NO3)2] stimulates this process, the EPS values being 7-29% higher than the values obtained during cultivation in standard conditions. The maximum accumulation of sulfated EPS was detected on the 18th day, reaching the value of 46.00 mg/l, which constitutes approximately 85% of the total acid EPS accumulated. After the 18th day of cultivation, there is a gradual decrease in the content of sulfated EPS. As a result of the research, we can say that the maximum accumulation of total acidic and sulfated exopolysaccharides occurs earlier (18th day), than in the case of the reference sample (22nd day).

# ANTIFUNGAL ACTIVITY OF MICROALGAE ISOLATED FROM THE WATER OF "LA IZVOR" LAKE

Turcan O., Sirbu T.
*Institute of Microbiology and Biotechnology*
e-mail: turcanolga2019@mail.ru

CZU:582.26/.27:579.26 https://doi.org/10.52757/imb22.40

Currently, the attention of researchers is directed towards microalgae and cyanobacteria due to their use as an alternative source of antibiotics. Among the first isolated antimicrobial compounds is chlorelin, from *Chlorella sp*. which is a mixture of fatty acids that inhibits the growth of both gram-positive and gram-negative bacteria. Eicosapentaenoic acid, hexadecatrienoic acid and palmitoleic acid isolated from *Phaeodactylum tricornutum* have been shown to possess antimicrobial activity against *Staphylococcus aureus* gram-positive strain. Thus, the aim of the research was to determine the antifungal activity of 8 strains of microalgae, isolated from the lake "La Izvor".

The cultures were isolated by inoculation on liquid and solid mineral nutrient media. Hydroalcoholic extracts (60-70%) from microalgae biomass were used to determine the antifungal activity against phytopathogenic cultures of fungi.

Thus, the experiments showed that microalgae strains have antifungal activity against the tested cultures of pathogenic fungi, especially *Oscillatoria acutissima* and *Spirulina major* showed a clear inhibitory effect (diameter of inhibition zone more -24mm and 25mm, respectively) against *Alternaria alternata.* Biomass extracts of *O. planctonica, O. brevis (*diameter of the inhibition zone of 40 mm) *O. acutissima* and *Chlorella vulgaris,* have shown an inhibitory effect on the growth of *Aspergillus niger* and *Botrytis cinerea*. An inhibitory action on the growth of the pathogenic fungus *Fusarium solani*, have presented extracts from *O. planctonica, O. acutissima, S. major, Anabaena variabilis* and *Nostoc verrucosum.*

*The research was funded by NARD within project 20.80009.7007.09.*

# CHARACTERIZATION OF MICROBIAL CONSORTIUM ISOLATED FROM LANDFILL SOIL POLLUTED WITH POLYETHYLENE

Vorona V., Rastimesina I., Postolachi O., Mamaliga V., Voinescu A.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: valentina.imb@yahoo.com

CZU:579.26:631.46 https://doi.org/10.52757/imb22.41

It is well known that large quantities of non-recyclable plastic, namely low-density polyethylene (LDPE), represent a major pollution problem in the environment, thus a solution would be its degradation through the adhesion and accumulation of microorganisms' consortia on the polyethylene surface. The microbial complexes that are involved in the decomposition of non-recyclable plastic were created in the polluted soil collected from the landfill situated near Slobozia-Dusca village. Consortia of microorganisms isolated from soil LDPE-treated under the aerobic conditions were inoculated into the liquid mineral salt media MSM 2 and MSM 4 to obtain enrichment cultures. MSM 2 medium was favoring the growth of micromycetes and MSM 4 medium – the growth of bacteria. Four microbial consortia were obtained.

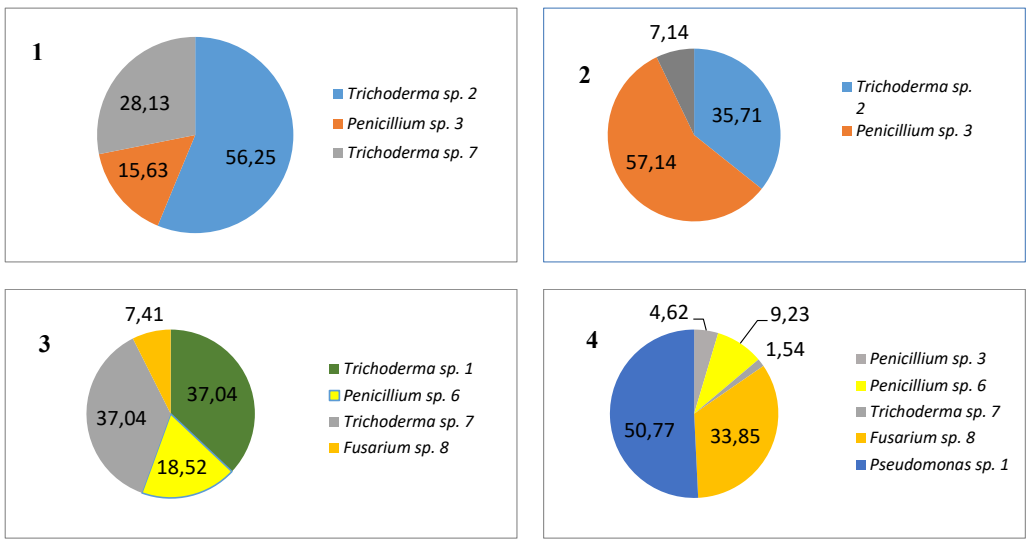



**Figure 1.** Composition of consortia isolated from soil treated with LDPE under aerobic conditions.

Analyzing the consortia of microorganisms obtained, which includes mycelial fungi and bacteria, we observed that the fungi are predominant, for the most part representatives of the *Trichoderma* spp., *Penicillium* spp., and *Fusarium* spp., while for bacteria only the genus *Pseudomonas* spp. was identified (fig. 1.). The results demonstrate that after 100 days of cultivation, the microorganisms in the consortia retain their viability, the titer being from $7.00 \times 10^6$ CFU/mL to $26.00 \times 10^6$ UFC/mL.

In conclusion, that complexes of microorganisms isolated from polluted soil treated with LDPE can use plastic as a potential source of carbon and/or energy.

*Acknowledgments. This work was funded by NARD of the Republic of Moldova within State Program Project 20.80009.7007.03 "Microbial tools for degradation of non-recyclable plastics waste."*

**ANTAGONISTIC PROPERTIES OF RHIZOBACTERIA IN RELATION TO PHYTOPATOGENS OF WHEAT**

Zakiryaeva S.[1], Atadjanova Sh.[1], Khomidjonova S.[2], Shakirov Z.[1]
[1]*Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan, Uzbekistan*
[2]*National University of Uzbekistan named after Mirzo Ulugbek, Uzbekistan*
e-mail: szakiryaeva@gmail.com

CZU:579.264:579.8+632.4:633.1 https://doi.org/10.52757/imb22.42

Plant protection from phytopathogens is becoming an increasingly severe problem in modern agriculture and agricultural microbiology. Today, there is a trend around the world towards an increase in the number of phytopathogenic micromycetes in agricultural soils due to the unreasonable introduction of chemical ameliorants into the soil and mismanagement in agricultural practices. This trend leads to the development of various diseases of agricultural plants, their death, a decrease in productivity, as well as a decrease in soil fertility. Therefore, in recent years, the microbiological method of plant protection has become widespread.

The aim of our research was to study the antagonistic activity of rhizobacteria in relation to wheat phytopathogens. The objects of research were local active strains of phosphate and potassium-mobilizing wheat rhizobacteria of the genera *Rahnella*, *Enterobacter*, *Pantoea*, *Pseudomonas*, *Bacillus* and phytopathogenic wheat fungi. As test cultures in determining the antagonistic properties of rhizobacteria, 6 strains of phytopathogenic fungi (*Fusarium graminearum*, *F. oxysporum*, *F. tricinctum*, *F. avenaceum*, *Bipolaris sorokiniana*, *B. spicifera*) of wheat were taken from the collection of the Institute of Genetics and Experimental Plant Biology of the Academy of Sciences of the Republic of Uzbekistan. The antagonistic activity of rhizobacteria against phytopathogenic wheat fungi was studied by the well diffusion method on Czapek's medium.

We have studied the antagonistic activity of rhizobacteria against phytopathogenic fungi causing various wheat diseases. The data obtained by us showed that 22 strains of rhizobacteria, of all tested, exhibit strong antagonistic activity in relation to one or another test culture. Thus, the *Pseudomonas* spp. 10R exhibited varying degrees of antagonistic activity against all test cultures of phytopathogens. Whereas other strains of *Bacillus cereus* 7R and *Pseudomonas kilonensis* 9R were active only against 3 phytopathogens - *F. graminearum*, *B. sorokiniana* and *B. spicifera*, the inhibition zones was 90%. The strains of the species *Rahnella aquatilis*, only 2 strains No. 10 and 14 showed antagonistic activity against 2 test cultures - *F. graminearum* and *B. spicifera*, the zones of inhibition was 100%. The bacterial species *Enterobacter clocae*, strain No. 7 had a stronger antibiotic property. Thus, the zone of inhibition in relation to *B. sorokiniana* was 100%, *F. graminearum* and *F. oxusporum* - 90%, *F. tricinctum* - 80%. The *B. megaterium* 22R strain was also active against two phytopathogens, *F. graminearum* and *F. avenaceum*, the zone of inhibition was 80%. The *B. subtilis* 24R strain showed antagonistic activity against 5 test objects, although the zones of inhibition were insignificant, so the zone of inhibition against *F. graminearum* was 16 mm, *F. tricinctum* and *B. sorokiniana* - 11 mm, *F. avenaceum* - 20 mm, on *B. spicifera* - 22 mm. The *P. agglomerans* 1R strain showed antagonistic activity only against *F. graminearum* (8 mm), *F. tricinctum* (10 mm), and *F. avenaceum* (11 mm).

Thus, under laboratory conditions, the antagonistic activity of wheat rhizobacteria in relation to wheat phytopathogens was determined. *Pseudomonas* spp. 10R, *B. cereus* 7R, *P. kilonensis* 9R, *R. aquatilis* 10, 14, *E. clocae* 7 and *B. megaterium* 22R bacterial strains exhibited the highest antagonistic activity against phytopathogenic wheat fungi among all the studied strains.

**METAL REMOVAL FROM ERBIUM -CONTAINING WASTEWATER USING ARTHOSPIRA PLATENSIS**

Yushin N.[1,2], Zinicovscaia I.[1,3,4], Cepoi L.[5], Chiriac T.[5], Rudi L.[5] and Grozdov D.[1]

*[1] Department of Nuclear Physics, Joint Institute for Nuclear Research, 141980 Dubna, Russia*
*[2] Doctoral School Biological, Geonomic, Chemical and Technological Science, State University of Moldova, MD-2028 Chisinau, Moldova*
*[3] Department of Nuclear Physics, Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, 077125 Bucharest, Romania*
*[4] Laboratory of Physical and Quantum Chemistry, Institute of Chemistry, MD-2028 Chisinau, Moldova*
*[5] Laboratory of Phycobiotechnology, Institute of Microbiology and Biotechnology, MD-2028 Chisinau, Moldova*
*Ynik_62@mail.ru*

CZU:628.35:582.232 https://doi.org/10.52757/imb22.43

Erbium belongs to rare earth elements critical for industry, especially nuclear technology. Cyanobacteria *Arthospira platensis* was used for Er(III) removal from wastewater by applying biosorption and bioaccumulation processes. The influence of pH, Er(III) concentration, contact time and temperature on the biosorption capacity of *Arthospira platensis* was determined. The optimal conditions for Er(III) removal were defined as pH 3.0, time 15 min and temperature 20 ˚C, when 30 mg/g of Er(III) were removed. The kinetics of the process was better described by the pseudo-first-order model, while equilibrium fitted to the Freundlich model. In bioaccumulation experiments, the uptake capacity of biomass and Er(III) effect on biomass biochemical composition were assessed. It was shown that Er(III) in concentrations 10–30 mg/L did not affect the content of biomass, proteins, carbohydrate and photosynthetic pigments. Its toxicity was expressed by the reduction of the lipids content and growth of the level of malonic dialdehyde. Biomass accumulated 45–78% of Eu(III) present in the cultivation medium. Therefore, *Arthospira platensis* can be considered as a safe and efficient bioremediator of erbium contaminated environment.

# EVALUATION OF ORGANIC WASTE APPLICATION EFFICIENCY FOR SYNTHESIS OF CHOLESTEROL OXIDASES

Zhukouskaya L., Semashko T., Muntsianava M.
*Institute of Microbiology of the National Academy of Sciences of Belarus, Belarus*
e-mail: mila_zhu@mail.ru

CZU:579.6 https://doi.org/10.52757/imb22.44

One of the major challenges in biothechnology is the rational disposal of enormous volumes of organic wastes released by the global industrial sector. Molasses and glycerol are referred to widely distributed types of waste materials.

Molasses is a by-product of sugar refining. Its yield is approximately 4,6% by weight of the processed sugar beet mass. Molasses is distinguished by unpleasant odor and taste making it largely inedible for humans.

Glycerol is one of the main components of organic waste discharged by manufacturers of biofuel, foodstuffs and cosmetics. Significant amounts of glycerol are generated during commercial distillation of alcohol in rectifying columns, bioethanol fermentation from vegetable feedstock and in biodiesel process where glycerol acts as the principal by-product. Each gallon of produced biodiesel is known to be accompanied by output of around 0,3 kg of crude glycerol amassing annually in Europe the stockpile over 60 thousand tons.

In this regard research of methods to utilize wastes containing molasses and glycerol is a vital prerequisite for launching eco-safe biotechnologies.

Aim of the study was to estimate efficiency of organic waste (glycerol and molasses) application on growth of mycelial fungi and synthesis of cholesterol oxidases (ChO).

Earlier we performed screening of new mycelial fungal strains synthesizing ChO and selected the cultures showing the highest level of enzyme production.

It was found in the course of the study that supply into the nutrient medium of 2,5-5,0% molasses increased ChO generation level in *A. aliaceus* F and *A. aliaceus* by 1,8 times, equaling 0,08 U/ml and 0,076 U/ml, respectively. Biosynthesis of enzyme by *A. niger*, *P. kapuscinskii* and *P. chrysogenum* remained on the control level (0,036-0,048 U/ml). Decline of enzyme biosynthesis 2-fold (0,022 U/ml) occurred in *P. roquefortii* culture. As to growth parameters of these fungi, it should be noted that in case of supplementation of molasses biomass accumulation either stayed on the control level (6,06 mg/ml) or rose twice (14,98 mg/ml).

When 2,5-5,0% glycerol was fed to the nutrient medium ChO production fell 2-10 times in *A. aliaceus* (0,004-0,02 U/ml) and was not evident at all in *A. aliaceus* F and *A. niger*. Cultivation of *P. canescens*, *P. kapuscinskii* and *P. roquefortii* with 2,5-5,0% glycerol decreased ChO biosynthesis level by 1,25-1,75 times (0,012-0,044 U/ml). Supply of 5,0% glycerol into the cultural medium with *P. chrysogenum* kept biosynthetic capacity of the strain on the control level (0,028 U/ml). Analysis of biomass accumulation showed that upon supply of glycerol in tested amounts concentration of biomass was comparable to the control (4,23 mg/ml) or rose twice (8,87 mg/ml).

Thus, it was established that in some strains of mycelial fungi producing ChO it is possible to substitute glycerol and molasses as carbon sources in the nutrient media achieving the same level of enzyme biosynthesis (*A. niger*, *P. kapuscinskii*, *P. chrysogenum*) or even increasing it 1,8 times (*A. aliaceus* F, *A. aliaceus*).

*The research was conducted within the framework of the project B21APM-021 financially supported by Belarusian Republican Foundation of Basic Investigations.*

BLUE

# Biotechnology

Aquaculture, Coastal and Marine Biotehnology

# MODIFICATION OF THE YOLK SAC IN CARP LARVAE DEPENDING ON THE VARIETY OF ENVIRONMENTAL TEMPERATURES

Balacci S., Balan I., Buzan V.
*Institute of Physiology and Sanocreatology, Republic of Moldova*
e-mail: sergiobalacci@gmail.com

CZU:597.551.2:591.3 https://doi.org/10.52757/imb22.45

The purpose of the research is to highlight the effects of moderate intensity temperature stress on the consumption of nutrients of the yolk sac of fish larvae. The temperature values that could be applied in order to stimulate the growth of adaptive capacities and the resistance of carp larvae to the unfavorable action of the environment were determined. As final results is presented the possibility of using the environmental factors applied to fish as a model for the application of ecological factors on homeothermic animals, in particular, on agricultural animals.

The10 days study was carried out on carp larvae (Cyprinus carpio) divided into 3 groups (aged 1, 2 and 3 days), consists of 4 experimental sublots, in which temperatures of 9, 12, 15 and 20ºC were applied. The batch in which the water temperature was 20ºC (identical to the water temperature during incubation) served as a control. The experiment was carried out in 3 liters vessels, 500 larvae per liter density. The adaptation period to the tested temperatures was 1 hour. Starting from day 3, the carp larvae were fed abundantly with live zooplankton. The yolk sac parameters (length and height) were monitored on the 1st, 3rd, 5th, 7th and 10th day after applying the thermal factor. The dimensions of the yolk sac were determined using a microscope equipped with a lens to evaluate the linear size of the studied object.

The obtained data demonstrate that the thermal variations of the water act differently on the absorption rate of the yolk sac in carp larvae aged 1 day. The lower the temperature, the slower it is absorbed. At the temperature of 9°C, the length of the yolk sac in the larvae subjected to the action of the temperature for 10 days decreased compared to it in the larvae subjected to the action for 1 day by 0,46mm (15,49%) and constituted 2,51±0,08mm versus 2,97±0,08mm. At the temperature of 12°C, the yolk sac length decreased by 1,04mm (64,26%) and was 1,87±0,07mm compared to 2,91±0,05mm. When the larvae are exposed to higher temperatures (15 and 20°C) the yolk sac is completely absorbed.

The same tendency is manifested in the larvae of group II. The size of the yolk sac in 2-day-old larvae subjected to the action of the thermal factor is smaller compared to the size of the yolk sac in 1-day-old larvae at all temperatures studied and throughout their application. The yolk sac at the larvae subjected to the action of the temperature of 9°C for 10 days decreased relative to it at the larvae subjected to the action for 1 day by 0,89mm (32,84%), at the temperature of 12°C it shrank by 1,77mm (68,34%). At the same time, at higher temperatures (15 and 20°C) the yolk sac is fully absorbed. Moreover, it should be noted that at 15°C the yolk sac is absent in the sublot in which the temperature was applied for 7 days compared to it in the 1-day-old larvae whose length was 1,62±0,11mm. When applying temperatures of 20°C for 5 days (which corresponds to the age of the larvae of 7 days), the yolk sac is practically not noted and corresponds to the data of the specialized literature. It is worth mentioning that the absorption rate of the yolk sac in group II is higher compared to the absorption rate in the 1-day-old carp larvae experiment.

The yolk sac at carp larvae of group III is preserved during all periods of application of the temperature of 9°C. At the temperature of 12, 15 and 20°C it is recorded only up to the duration of the application of the stressogenic factor of 3 days.

Thus, it can be mentioned that the application of low temperature on carp larvae leads to the retention of their development with the preservation of the yolk sac for a period of up to 10-12 days from birth. These results are more evident when applying the temperatures of 9 and 12°C in the groups where larvae at the beginning of the experiment were 1 day and 2 days old. So, by applying the thermal factor it is possible to direct the duration of the development period of carp larvae.

# PRESENCE OF *ACTINOBACTERIA* IN THE AQUATIC ECOSYSTEM OF THE "LA IZVOR" LAKE IN THE CHISINAU

Birsa M., Cebotari V., Burtseva S.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: maxim.birsa@imb.md

CZU:579.8:504.455(478-25) https://doi.org/10.52757/imb22.46

One of the effective ways to obtain new biologically active substances is the search for new strains of microorganisms - producers.

The most active antagonists among microorganisms are actinobacteria: of the 10,000 known antibiotics produced by microorganisms, about 70% are of actinomycete origin. Microorganisms, including actinobacteria, are of interest as enzyme producers, in particular, for the biocontrol of phytopathogenic fungi, as plant protection preparations, as well as for the biodegradation of plant residues, as a reservoir of infection in the soil for agricultural plants, etc.

The task of the research was to study the ability of strains of actinobacteria isolated from the biofilm of the "La Izvor" lake system to exhibit enzymatic activity, as well as to inhibit the growth of a number of phytopathogenic bacteria and fungi.

The objects of research were representatives of 8 genera of actinobacteria isolated from the biofilm of the lake system "La Izvor", Chisinau.

It was established that strain B 2.1 (genus *Actinoplanes*) has weak (+) amylase activity, medium (++) lipase and high (+++) catalase activity. Strain B 3.1 (genus *Frankia*) showed weak (+) catalase and moderate (++) amylase activity. In strain B 4.1 (genus *Geodermatophilus*) a weak (+) catalase activity was noted, as in strain B 6.1 (genus *Nocardia*). The representative of the *Micromonospora* strain B 5.1 was characterized by medium (++) catalase and weak (+) amylase activity. Four strains are representatives of the river. *Streptomyces* showed weak (+) amylase activity, 2 strains of this genus (B 8.1 and B 8.4) also had weak (+) catalase activity, and only strain B 8.1 showed medium (++) lipase activity.

Low antibacterial activity was observed in strain B 5.1 (genus *Micromonospora*) - the diameter of the growth inhibition zones of *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Clavibacter michiganensis*, *Erwinia carotovora* - 9.0 - 14.0 mm. From the strains of genus *Streptomyces*, only strain B 8.3 had the ability to inhibit the growth of 5 phytopathogenic bacteria with a zone diameter of 12.0-16.0 mm.

It was found the ability to inhibit the growth of the phytopathogen *Alternaria alternata* by the strains B 2.1, B 4.1, B 8.3, and B 8.4 (zone diameter - 15.0 - 20.0 mm). The growth of *Fusarium solani* was retarded by 6 strains (zones from 10.0 to 20.0 mm); *Fusarium oxysporum* - by 2 strains (zones 12.0-14.0 mm); and *Aspergillus niger* – by 4 strains (zones 12.0 - 16.0 mm).

Thus, the conducted studies have shown that new strains of actinobacteria isolated from the biofilm of the "La Izvor" lake system, Chisinau, which have shown amylase, catalase, and lipase activity, as well as having the ability to inhibit the growth of a number of phytopathogens, are of particular interest and can replenish the National Collection of Non Pathogenic Microorganisms.

*The research was funded out within the project 20.80009.7007.09 (NARD).*

## EFFECT OF LACTOBACILLI ON AUTOCHTHONOUS MICROFLORA OF FISH PONDS

Jurminskaia O., Shubernetsky I., Andreev N.
*Institute of Zoology, Republic of Moldova*
e-mail: ojur_aia@mail.ru

CZU:606:579.6 https://doi.org/10.52757/imb22.47

The abundance of bacterioplankton and bacteriobenthos in fish ponds is largely determined by three main factors: water temperature, fish stocking density and fertilizers used. As the water temperature rises, the intensity of the metabolic processes of the microbiota increases, and their number increases significantly. The greatest number of microorganisms is contained in the surface layer of bottom sediments. Intensive consumption of the oxygen by bacterioplankton and bacteriobenthos can lead to fish kills. The use of probiotics in aquaculture is of great interest: their influence on the immunostimulation of farmed fish, direct inhibition of pathogenic bacteria and improvement of pond water quality have been studied by many researchers.

In order to determine measures to improve water quality in fish ponds, the laboratory experiment with the probiotic *Lactobacillus acidophilus* was carried out in the conditions of the Laboratory of Hydrobiology and Ecotoxicology. In this experiment, water samples were collected from the fish ponds of the "Ghidrin-Falesti Fish Enterprise" in RM. The degree of water body organic pollution is characterised by the hydrochemical parameter $BOD_5$, which varied from 8 to 28 (mg/L $O_2$) in water samples of these fish ponds. In accordance with the "Regulations" in force in the Republic of Moldova (2013), $BOD_5$ values > 7 (mg/L $O_2$) correspond to water quality class V. Thus, in terms of the amount of organic substrate, the water in these ponds is a good habitat for heterotrophic microorganisms. As a result of the development of the scientific basis of the theory of fertilisation of fishponds by Soviet hydrobiologists (N. Arnold, G. Vinberg, V. Zhadin, A. Rodina, etc.) the following was revealed: when fish is raised in high stocking with artificial compound feed, the task arises to limit bacterial development, i.e. to manage bacterial processes in the fish ponds. The task remains relevant today.

The aim of the experiment was to test the ability of lactobacilli to survive in the bottom layer of a fish pond in comparison with autochthonous microflora. For this purpose, water samples from the Calugar, Girla and Fagadau ponds were divided into two aliquots: matrix (natural sample) and matrix + lactobacilli. Lactobacilli are non-pathogenic Gram-positive microorganisms with high enzymatic activity. In relation to oxygen, they are microaerophiles. By type of nutrition, they are chemoheterotrophs, using organic compounds as a source of energy and carbon. All aliquots were incubated at 22°C without aeration and also without access to light to minimise the photosynthetic activity of phytoplankton. After five days, each aliquot was inoculated (at the appropriate dilution) into Petri dishes on Tergitol 7 agar (without TTC) and incubated at 22°C. In sanitary microbiology, MRS agar is used for testing lactobacilli and cultivation is carried out at (30 - 35) °C. In our experiment, the aim was not to create the specific conditions for lactobacilli. We used Tergitol 7 agar, which contains lactose as opposed to MRS agar, which contains dextrose. To find out the ability to grow on Tergitol 7 agar and to determine the specific characteristics of the colonies, a *Lactobacillus acidophilus* culture was inoculated on this medium and cultivated under the same conditions as the test samples. The results of the experiment are presented below:

| | Fagadau | Girla | Calugar |
|---|---|---|---|
| Matrix on the day of sampling, $10^3$ CFU/mL | 3,3 | 3,2 | 2,5 |
| Matrix after 5 days, $10^3$ CFU/mL | 30 | 10 | 5 |
| Matrix + *L. acidophilus* after 5 days, $10^3$ CFU/mL | 4725 | 1805 | 2625 |

Thus, under oxygen-deficient conditions, the autochthonous microflora of fish ponds cannot withstand competition with lactobacilli, which are microaerophiles. If the results of the laboratory experiment are confirmed in real conditions, lactobacilli may be recommended for the suppression of autochthonous microflora, which are intensive oxygen consumers in summer biocenoses.

*The investigations were carried out within the framework of the bilateral project 2SOFT1.2/47 "Team up for healthy fish in aquaculture systems of the Prut river basin" implemented by the Institute of Zoology (Chisinau, Republic of Moldova) in collaboration with the Ion Ionescu de la Brad University of Agricultural Sciences and Veterinary Medicine (Iasi, Romania).*

# STUDY OF THE ENZYMATIC PROPERTIES OF FUNGI IN THE "LA IZVOR" AQUATIC ECOSYSTEM

Moldovan C.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: tina—92@mail.ru

CZU:574.5:582.28 https://doi.org/10.52757/imb22.48

The physiological adaptability of fungi and the multi-enzyme metabolic system is the basis of their amazing ability to develop in various environmental conditions, considered the engines of natural ecosystem restoration. They are natural decomposers of organic matter to absorb their nutrients, thus allowing recycling, mineralization and release of compounds for the community and ecosystems. Extracellular enzymes of fungal origin, both redox and hydrolytic, have been reported for various industrial and biotechnological applications, such as the medical, agricultural, pulp and paper, textile, detergent, food processing and biofuel industries; as well as bioremediation. In addition, fungal enzymes have a significant advantage over those derived from plants or animals due to their ease of handling, rapid production in low-cost media, higher yields, and catalytic activity.

The purpose of the research was to study the enzymatic properties (amylase, catalase, cellulase, lipase) of 93 strains of micromycetes representing the genera *Aspergilus*, *Penicillium*, *Trichoderma*, *Fusarium*, *Mucor*, *Rhizopus*, isolated from the "La izvor" aquatic ecosystem. Strains were isolated from water (35 strains), biofilm (28 strains) and silt (30 strains). Express tests were performed to determine the enzyme capacity: amylase, catalase, cellulase, and lipase. When determining the enzyme capacity, specific indicators were used for each enzyme: amylase - Lugol's solution, catalase - $H_2O_2$ (3%), cellulase - carboxymethylcellulase and Congo red, lipase - Tween 80. The enzyme activity was assessed as: (+++) – high; (++) – average; (+) – weak; (-) – missing.

As a result of the research on the strains isolated from the water, it was found that, in 12 strains, the activity of catalase was at an average level (++), in 11 strains - weak (+), and in 12 strains this activity was missing (-). Amylase, lipase, and cellulase activities were also weak in most strains tested. Only 4 strains registered an average enzymatic activity (++) of the 3 enzymes. In the rest of the strains, the activity of these enzymes is weak (+) or absent (-). The activity of amylase was not manifested in 10 strains, of lipase in 13 strains, and of cellulase in 12 strains. None of the tested strains showed medium-level activity of the studied enzymes, and medium-level enzymatic activity of 3 enzymes did - the strains isolated from water: A 12 and A 14.

In the strains isolated from the biofilm, the enzymatic capacity of the 4 enzymes was presented as follows, catalase activity: average (++) - 3 strains, weak (+) - 15 strains, missing (-) - 10 strains. Amylase activity: 2 strains – medium, 11 strains – weak, 15 – missing. Lipase and catalase activity in the tested strains was also weak or absent. None of the strains isolated from the biofilm showed moderate lipase and cellulase enzyme activity. Lipase activity was missing in 9 strains, and cellulase activity in 18 strains.

Thirty strains isolated from the silt were tested. From the 30 strains tested, catalase activity at a medium level (++) was recorded in 20 strains, at a weak level (+) in 8 strains and absent (-) in 2 strains. Amylase activity at a medium level - 18 strains, at a weak level - 10 strains, and missing in 2 strains. Lipase activity at a medium level - 18 strains, at a weak level - 9 strains, missing (-) in 3 strains. Cellulase activity: medium level - 6 strains, weak level - 19 strains, missing in 5 strains. Thus, we can state that from an enzymatic point of view the most active strains are the strains isolated from the silt. In 4 strains, the enzymatic activity of catalase, amylase, lipase and cellulase was recorded at medium level (++). These are strains N 5, N 7, N 12, N 14.

According to the obtained results, we can mention that the aquatic strains of micromycetes possess selective enzymatic properties. The most active ones possessing significant enzymatic properties (A 12 and A 14, N 5, N 7, N 12, N 14) were selected for further research.

*The research was funded out within the project 20.80009.7007.09 (NARD).*

# VIABILITY AND STABILITY OF LYOPHILIZED MICROMYCETES IN THE PRESENCE OF Cu AND ZnO NANOPARTICLES

Sirbu T., Turcan O., Timus I.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: tfsirbu@gmail.com

CZU:579.6:578.5 https://doi.org/10.52757/imb22.49

The publications of recent years demonstrate the effect of nanoparticles (NPs) on growth, morpho-cultural peculiarities, viability and biosynthetic processes in microorganisms. It was demonstrated that with the help of nanoparticles introduced into the microorganism cultivation medium, their morphological characteristics can be modified and biosynthetic processes stimulated, thus obtaining the expected microbial product of a higher quantity and quality.

This material presents the results obtained in the study of the viability and stability of 20 micromycetes from the genera *Aspergillus*, *Trichoderm*a, *Penicillium* lyophilized in the presence of Cu NPs and ZnO NPs. Morpho-cultural peculiarities and antifungal activity were studied. Phytopathogens were used as test cultures: *A. niger*, *A. alternata*, *B. cinerea*, *F. solani*, *F. oxysporum*.

It was found that the supplementation of Cu or ZnO NPs in the culture medium acts individually and insignificantly on the morpho-cultural particularities of the studied micromycetes. In some cultures, changes related to the order of color, growth rate and colony sporulation were observed, and in others insignificant stimulations or decreases in colony growth were recorded. The viability of strains grown on agar media supplemented with NP after lyophilization and storage in a lyophilized state, in most cases, varies within the limits of ± 2-4%, but there can also be stimulations up to 10%, compared to the control variant. ZnO NP acts more beneficially on the biosynthetic properties, stimulating the antifungal activity of *Penicillium* and *Trichoderma* strains, compared to the tested phytopathogens, from 2% to 16%, compared to the control.

ZnO NPs in a concentration of 0.1 mg/l supplemented in the lyoprotective medium have a beneficial effect on viability. Thus, it stimulates the viability of strains of the genus *Aspergillus* by 4 - 12.5%, of strains of the genus *Trichoderma* by 10-14.7% compared to the control, and compared to strains of the genus *Penicillium* it is neutral (± 1%/M), but after 1 year of storage in a lyophilized state, the viability of the studied strains is at the level of the control variant. ZnO nanoparticles supplemented in the protective medium can modify the antifungal activity of micromycetes of the genus *Trichoderma* and *Penicillium* against some phytopathogens. In most cases, it contributes to the stimulation of antifungal activity against the phytopathogens tested by 4-30%. Changes in the morpho-cultural characteristics of the studied cultures after lyophilization were not observed.

The action of Cu or ZnO NPs solutions as a rehydrator in the revitalization of micromycete strains is different depending on the concentration used and the tested culture. The rehydration of the strains with the Cu MPs solutions in the concentration of 0.001 mg/ml contributes to the stimulation by 1 - 4% of the viability, and the rehydration with the nanosolution of ZnO in the same concentration, decreases by 5-11% the viability of micromycetes, compared to the control.

It was found that Cu Nsolution in revitalization of lyophilized strains of the genus *Penicillium* significantly stimulates antifungal activity against phytopathogens tested by 4.0 - 40%, and the revitalization of strains of the genus *Trichoderma* by 7.5 - 21.3%, compared to the control.

At the same time, Cu NPs solutions used to revitalize lyophilized micromycete strains do not change their morpho-cultural particularities, and ZnO nanoparticles solutions significantly change these particularities, thus contributing to the reduction of micromycete growth and development.

According to the presented results, we can conclude that Cu and ZnO NPs supplemented in the cultivation medium, the protection medium or the revitalization medium of lyophilized strains, act insignificantly on the growth, viability after lyophilization and storage, but significantly modify the biosynthetic properties, significantly stimulating the antifungal activity against the phytopathogens tested.

*The research was funded out within the project 20.80009.7007.09 (NARD).*

# AMYLOLYTIC AND CELLULOSOLITIC BACTERIA ON THE MOLDAVIAN SECTOR OF THE DNIESTER AND PRUT RIVERS IN 2019 – 2022

Shubernetsky I., Negru M., Jurminskaia O.
*Institute of Zoology, Republic of Moldova*
e-mail: i.subernetkii@mail.ru

CZU:579.266:574.63(478) https://doi.org/10.52757/imb22.50

The analysis of the long-term dynamics of any indicator of the biocenosis state is always of great scientific and practical interest. The aim of the presented work was to study the spatial and seasonal dynamics of the abundance of amylolytic and cellulolytic bacteria in the Dniester and Prut rivers for 2019-2022 in order to identify long-term average trends. Sampling was carried out during the vegetative period of the year (spring-summer-autumn) at the stations presented in Fig. 1 - 2. After data processing, it was found that the number of amylolytic bacteria in the Dniester River varied in the range from 0.08 to 1.76 thousand cells/ml. The clear trend of the maximum number of this physiological group of bacterioplankton is recorded at Erjova station, located in the upper sector of the Dubossary Reservoir on the Dniester River (Fig. 1, a). In the Braniste-Giurgiulesti sector of the Prut River, the number of amylolytic bacteria varies within a narrow range: 0.50-0.97 thousand cells/ml (Fig. 1, b).

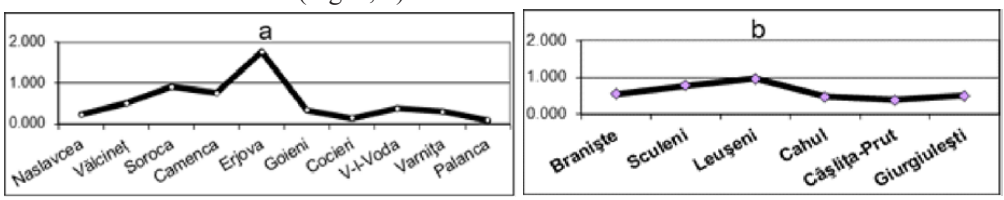



**Figure 1.** Spatial dynamics of amylolytic bacteria abundance (*N*, thousand cells/ml) in the spring season 2019-2022 in the rivers Dniester (a) and Prut (b)

The spatial dynamics of cellulosolitic bacteria in the Dniester River over the analysed period shows a trend characteristic for point pollution of the water body (Fig. 2, a): peaks of values can be caused to the inflow of un-normatively treated wastewater into the river (Soroca and Varnita stations) or secondary contamination - the dying off of aquatic vegetation (Cocieri station in the Dubossary Reservoir). In the Prut River, a insignificant increase of cellulosolitic bacteria abundance is registered in the Sculeni - Cahul sector (Fig. 2, b).

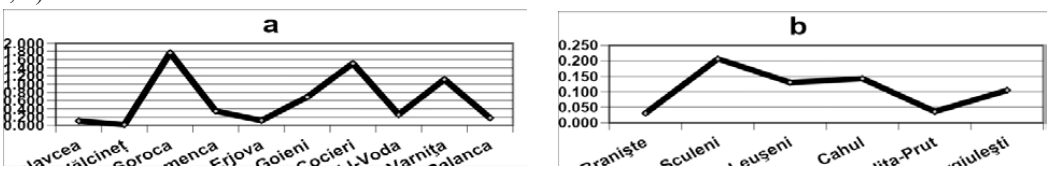



**Figure 2.** Spatial dynamics of cellulosolitic bacteria abundance (*N*, thousand cells/ml) in the summer season 2019-2022 in the rivers Dniester (a) and Prut (b)

In both rivers, the pronounced interannual abundance fluctuations of these physiological groups of bacteria are periodically observed, which noticeably changes the average long-term indicators (Fig. 3).

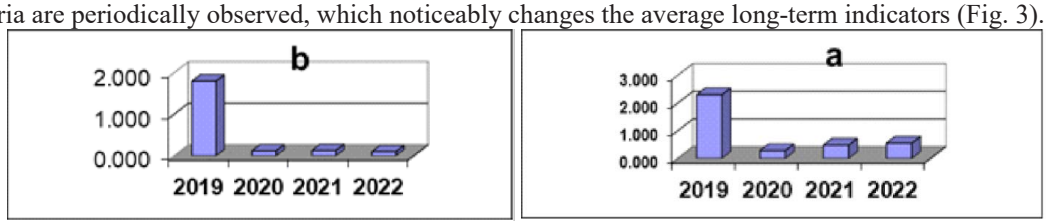



**Figure 3.** Interannual dynamics of amylolytic bacteria abundance (*N*, thousand cells/ml) in the rivers Dniester (Soroca station) and Prut (Braniste station)

*The investigations were carried out within the framework of the institutional applied research project AQUASYS in 2019 and the State Programme project 20.80009.7007.06 AQUABIO in 2020 - 2022.*

# USE OF THE EXTRACTS OF CYANOBACTERIA *CALOTHRIX MARCHICA, NOSTOC HALOPHYLUM* AND *SPIRULINA PLATENSIS* FOR THERAPEUTIC PURPOSES

Trofim A., Bacalov I., Zosim L., Bulimaga V., Turcan O.
*State University of Moldova, SRL Phycobiotechnology*
e-mail: alinatrofim@yahoo.com

CZU:633.88+615.322 https://doi.org/10.52757/imb22.51

One of the current problems of society is the establishment of an alternative natural treatment for diabetes that is harmless to human health.

The aim of our work was to study the level of glucose and the concentration of insulin in experimental alloxan diabetes on the background of the administration of the extracts from cyanobacteria to an experimental group of laboratory rats.

In the present study, the influence of the action of the alcoholic extracts of cyanobacteria *Calothrix marchica*, *Nostoc halophylum* and *Spirulina platensis* on the activity of the endocrine pancreas in the case of experimental diabetes was monitored.

The research was conducted on a group of 48 rats who were given alloxan in order to provoke the classic symptoms of type II diabetes.

As a rule, 2-3 days after the injection of the diabetogenic agent, the glucose samples were analyzed and thus the onset of the disease in mammals is determined. Most of the time, diabetes is confirmed at the end of the experiments, when the blood is collected for various tests, including blood sugar. At the onset of the disease, extracts from the cyanobacteria *Calothrix marchica, Nostoc halophylum* and *Spirulina platensis* were administered orally through the liquid consumed by the rats in the form of liquid nutritional supplements.

During the research, a considerable increase in blood sugar was observed in the group with experimental diabetes in relation to the control samples.

As a result of the research, it was found that the glycemia in control subjects is 5.5 ± 0.62 mmol/l, and in the group with experimental diabetes - 16.9 ± 1.75 mmol/l. An important aspect is observed in the group where the *Calothrix marchica* supplement was administered on the background of alloxanic diabetes, a decrease in blood glucose level up to 8.8 ± 0.94 mmol/l is highlighted. A slightly weaker hypoglycemic effect was observed in the group where *Nostoc halophylum* and *Spirulina platensis* were administered on the background of experimental diabetes – 10.5 ± 1.88 mmol/l; 9.1±1.17 mmol/l.

In conclusion, the use of alcoholic extracts of the cyanobacteria *Calothrix marchica*, *Nostoc halophylum* and *Spirulina platensis* demonstrated a beneficial effect in the treatment of type II diabetes and can be recommended as nutritional supplements in this case.

*The given research was carried out within the project "Determination of Bioactivity and Antimyeloma Properties of Various Cyanobacteria" project number- 22.80013.5107.2TR*

# Yellow Biotechnology

Food Biotechnology,
Food Science

# THE ROLE OF INDIVIDUAL MEMBERS OF THE FAMILY STREPTOCOCCACEAE IN THE HEALTH OF THE ORGANISM UNDER DIFFERENT TYPES OF NUTRITION

Bogdan V.
*Institute of Physiology and Sanocreatology, Republic of Moldova*
e-mail: victoriabogdan@gmail.com

CZU:579.63:613.2 https://doi.org/10.52757/imb22.52

*Streptococcus* bacteria, genera Streptococcus, Lactococcus and Enterococcus, are always isolated from the intestinal contents of children and young mammals. They are mostly beneficial for the host organism and their quantitative and species composition depends on the health statute. The objective of our studies was to continue the work on elucidating the role of the genus Enterococcus in the health of the organism under different types of nutrition.

The studies were performed in three stages: 1) determination of the frequency of Enterococci in the digestive tract of humans and animals; 2) establishment of their number in the rectum content of healthy children and adults of different ages (1-3 and 20-80 years) and 3) determination of quantitative and qualitative indicators of Enterococci in the intestines of white rats fed diets with different contents of proteins, fats and carbohydrates.

182 strains of streptococci isolated from human and animal intestines, after identification, 135 belonged to the genus Enterococcus (this constituted 74.14 %). Regarding their numbers, it was found that in healthy children in the postnatal period of life (1-3 years) the number of Enterococcus was 5.53±0.12 to 5.92±0.11 log/g, and in children with intestinal disorders - 8.45±0.18 to 8.46±0.20 log microbial cells in 1 g of the intestinal content. Differences in the number of enterococci in the intestine were also noted in adults, but in people with a healthy status of 20 to 50 years it was in the range of 7.54±0.16 to 7.90±0.11, and with a pathological health status - enterococci were found in the range of 9.38±0.22 and 9.60±0.22 log/g.

In people over 50, the total quantitative values of streptococci in the intestine increased, being in the range of 8.20±0.13 - 8.77±0.17 in healthy and 9.77±0.20 - 9.88±0.23 log microbial cells in 1 g of intestinal content - in patients. In these individuals, the proportion of enterococci - decreased, amounting to only 35.39%. Differential studies confirmed the prevalence of *Enterococcus fecalis* species in relation to *Enterococcus faecium* species by a factor of 3.5 (were 77.77 and 22.23 %, respectively).

Further we studied the quantitative and qualitative indicators of *Enterococcus fecalis* in the intestines of white rats, against the background of the application of diets with different compositions of proteins, fats and carbohydrates. The experiments were carried out using four versions of diets (1) including the above components in 8, 35 and 37%; 2) 11, 29 and 60%; 3) 12, 27 and 61% and 4) 14, 25 and 61%, which were tested in four experimental groups. The first was the control (I), and the second, third and fourth groups (II, III and IV) were experimental, and the data obtained are reflected in the table.

**Table 1. Quantitative indicators of enterococci in the contents of the rectum of rats on which different food diets were tested**

| Group | Nr of bacterial cells in 1 g of intestinal contents, log | | Difference, % | |
|---|---|---|---|---|
| | Start of the experiment | End of the experiment | Start of the experiment | Control group |
| I | 5,11±036 | 8,65±0,42 | + 69,27 | |
| II | 5,50±0,39 | 6,58±0,48 | +19,63 | - 23,93 |
| III | 5,67±0,41 | 6,63±0,39 | + 16,93 | - 23,35 |
| IV | 5,23±0,22 | 6,17±0,41 | + 17,97 | - 28,67 |

Based on the data in the table, we can note that the ration tested in the control group (I) had a negative effect on the reproduction process of the determined microbial representatives. This led to intensive development of facultative microorganisms of Enterococcus genus, which was confirmed by their quantitative indices at the end of the experiment, which were higher (by 69.27 %) in comparison with the beginning of the experiment. But at the same time, we note that the food rations tested in the experimental groups had a positive impact, and this is confirmed by the number of studied bacteria, which in groups II, III and IV was lower by 23.93; 23.35 and 28.67%, respectively.

# ISOLATION, CHARACTERIZATION AND APPLICATION OF ACETIC ACID BACTERIA FROM LOCAL WINE PRODUCTS

Boistean A.[1], Chirsanova A. [1], Sturza R. [1], Gaina B. [2]
*1-Technical University of Moldova, Republic of Moldova*
*2- Academy of Sciences of Moldova, Republic of Moldova*
e-mail: alina.boistean@toap.utm.md

CZU:579.66:579.22:663.2 https://doi.org/10.52757/imb22.53

Recently, interest has increased in the problem of nutrition and the production of natural products in the Republic of Moldova, including fermented products, the production of which requires local and active acetic acid bacteria (AAB). Agriculture and specifically wine are a business card of our country, internationally recognized. But AAB can play not only a negative role, but also a positive one for the processing of agricultural crops. The main goal of this work was to isolate a pure culture of AAB from local wine products for further use in production of DOP or IGP products.

AAB are a group of Gram-negative bacteria which oxidize sugars or ethanol and produce acetic acid during fermentation. They belong to the family *Acetobacteraceae*, which includes several genera and species. Currently, they are classified into nineteen genera. AAB are widespread in nature and play an important role in the production of food and beverages. These bacteria are also used in the production of other metabolic products, for example, gluconic acid, L-sorbose and bacterial cellulose, with potential applications in the food and biomedical industries.

Acetic acid bacteria live in greater or lesser numbers on all agricultural products. But for the study, unprocessed chemically white grapes of the Nova variety (Călărași district) and products of its processing (white wine, white wine with high acidity pH=2.89, wine must fermented to vinegar, laboratory production) and wine vinegar (unfiltered and unpasteurized) were taken for the study. The primary task was to isolate active and high-performance AAB. The isolation of pure cultures of acetic acid bacteria was carried out using conventional methods on three dense differential diagnostic media: GYC (yeast extract -10 g/L, $CaCO_3$-10 g/L, glucose - 3 g/L, agar - 15 g/L); RAE (glucose - 4g/L, peptone - 1g/L, yeast extract -1g/L, citric acid x $H_2O$ - 0.15g/L, $Na_2HPO_4$x $2H_2O$-0.34g/L, glacial acetic acid -1 mL/L, $C_2H_5OH$ 96% - 1 mL/L); Hoyer's ($(NH_4)_2SO_4$ -1 g/L, $K_2HPO_4$ -0.1g/L, $KH_2PO_4$ -0.9 g/L, $MgSO_4$ -0.25 g/L, $C_2H_5OH$ 96% -30 mL/L, FeCl3 1% - 0.5 g/L, $H_2O$ dist.). The media were manufactured and certified at the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova. Cultivation, storage and study of bacteria was carried out in the laboratories of the Faculty of Food Technologies, UTM, in a thermostat at a temperature of 27±1 °C for 3-5 days. The identification of isolates was carried out according to morphological, cultural, physiological and biochemical characteristics (gram stain, catalase test, KOH test) taking into account the properties characteristic of AAB. To confirm the belonging to the Acetobacter genus of the isolated bacteria, the real-time PCR method was used for detection by comparison of DNA. In our research molecular detection of AAB has been done using. For everyone Detection Kit B Acetics Screening ‘’, PIKA Weihenstephan GmbH, which contains all the materials necessary for this determination. The qPCR reaction was performed according to the manufacturer's protocol (#2401-15 4eTM for everyone Detection Kit B Acetics Screening User Guide).

In this study, a new strain of acetic bacteria with valuable biotechnological properties was isolated from native raw materials. Following the performance of biochemical tests and the application of the RT-PCR method, it was definitely established that the isolated strain belonged to the *Acetobacter* genus. The strain *Acetobacter aceti CNMN-AcB-01* was deposited in the National Collection of Nonpathogenic Microorganisms within the Institute of Microbiology and Biotechnology. Following the testing of the strain *Acetobacter aceti CNMN-AcB-01* in industrial conditions at the company "V. DEVELOP" SRL, the practical interest of its use in the production of domestic wine vinegar was found.

*The research was funded by State Project 20.80009.5107.09 “Improving of food quality and safety through biotechnology and food engineering”, running at Technical University of Moldova.*

# EVALUATION OF THE PRESENCE OF NITROFURANS IN MEAT AND CHICKEN EGGS

Burlacu S., Stici V., Enciu V.
Technical University of Moldova, Republic of Moldova
e-mail: svetlanaburlaku24@gmail.com

CZU:664.8.037.1:637.4/.54 https://doi.org/10.52757/imb22.54

***Key words:*** *poultry meat, egg, nitrofurans residues, contamination, monitoring.*

**Introduction**

The nitrofurans are antimicrobial drugs that have been widely used as veterinary therapeutics or feed additives for treating bacterial diseases in cattle, swine and poultry production. Furazolidone, furaltadone, nitrofurazone and nitrofurantoin are veterinary drugs that belong to the nitrofuran group, which have been used in the treatment of infections caused by *Escherichia coli* and *Salmonella* in pigs, poultry and fishes. The nitrofurans are quickly metabolized and are not detected after few hours from their administration. Otherwise, nitrofuran metabolites remain during months as residues bound to tissue proteins [2].

It has been demonstrated that a proportion of the bound residues of furazolidone and furaltadone possess intact side-chains which have molecular characteristics in common with the parent compounds [2]. These side-chains can be released from the bound metabolites under mildly acidic conditions such as may occur in the stomach of the consumer. It has been suggested that furazolidone sidechain, 3-amino-2-oxazolidinone (AOZ), can be metabolized into-hydroxyethylhydrazine, which is a mutagenic and carcinogenic compound [7].

Nitrofuran antibiotics were banned within the European Union (EU) due to the toxicological hazard for human consumers, concerns over their carcinogenicity and mutagenicity provoked by these drugs and should not be used in food-producing animals or be present in foods produced in, or imported into, the EU. The all compounds were put into the Annex IV of the European Union Directive no. 90/2377/EC in 1993 and 1995 [1-2], currently approved in the European Union Regulation no. 2010/37/EC. However, nitrofurans can be illegally administrated to animals. During 2002-2003, nitrofuran residues were detected in poultry and aquaculture products imported to Europe from different countries and the residue of nitrofuran metabolite was detected in poultry meat from Portugal and pork meat from Italy and Greece [2].

The monitoring of residues of nitrofuran metabolites in the food chain it is a major focus in the international control of veterinary drug residues and constitutes a control and supervision tool for safe food production [1].

Sometimes, the identification of some drugs in food is possible only through the presence of their metabolites, presented most of the time in insignificant amounts. There are several analytical techniques for determining these residues, however, in the Republic of Moldova the most developed are the immunoenzymatic techniques with the use of putties from various manufacturers.

There have been many papers involving LC-MS or LC-MS/MS method for the determination of nitrofuran metabolites including AMOZ in various matrixes. These methods are sensitive and confirmatory, but the expensive instruments may not be available in every laboratory. Comparison with those instrumental methods, ELISA is a low cost and sensitive method capable of screening large amount of samples in a single test [8.]

Although the aforementioned analytical methods offer predominant accuracy and high-throughput screening capability, they are relatively resource demanding and multiple steps of these methods hinders their instant and filed applications. Accordingly, it still remains tremendous requirements to construct facile detection methods with satisfactory simplicity, speed and cost, results visible by naked eye, small sample volume requirement, shorter detection time, ease of mass production and portability [6].

**Materials and methods**

The examinations were carried out in 7 samples of broiler chicken meat and 8 samples of chicken eggs. Broiler chicken meat and eggs were purchased from stores from different farmers. The examinations were carried out in 7 samples of broiler chicken meat and 8 samples of chicken eggs. Broiler chicken meat and eggs were purchased from stores from different farmers.

**Apparatus.**

For the enzyme-linked immunoassay method, was used a "SUNRISE" model absorbance reader, for the LC/MS/MS method, was used the "Shimadzu" model chromatograph.

**Reagents and chemicals.**

Ridascreen kits from R-biofarm were used for the enzyme-linked immunoassay method. The standard solutions and all reagents used were of high purity. For the LC/MS/MS method only reagents of >99.9% purity.

**Test principle for the enzyme-linked immunoassay method.**

The basis of the test is the antigen-antibody reaction. The microtiter wells are coated with capture antibodies directed against anti-nitrofuran metabolites antibodies. The measurement is made photometrically at 450nm. The absorbtion is inversely proportional to the metabolite concentration in the sample.

**LC/MS/MS analysis.**

The LC/MS/MS system is composed of Liquid Chromatograph Shimadzu System (Shimadzu Coorporation, Japan) connected to a quadrupole mass spectrometer in eletrospray positive ionisation mode.

**Sample preparation for the enzyme-linked immunoassay method.**

The samples homogenise, prepare 10mM metabolite in dimethylsulfoxide directly before use. Mix 1g of the homogenized sample with 4 ml distilled water, 0,5ml 1M HCL and 100µl for the metabolite solution by shaking thoroughly.

The derivatization procedure was performed according to the protocol.

**Sample preparation for the LC/MS/MS analysis.**

A 1,0 g portion of sample was transferred to a 15 mL centrifuge tube. The samples were submitted to hydrolysis and derivatisation processes, by adding 40µL of internal standard mixture for the AOZ, AMOZ, AHD, SEM and DNSAH. The samples were centrifuged for 10 min. After centrifugation was transferred to a glass tube and let evaporate to dryness at 45 ◦C in an evaporation station. The residues were redissolved in acetonitrile–water and 0.1% acetic acid mixture and centrifuged for 5 min.

**Calibration curve.**

Calibration curve was prepared with blank samples which were fortified with a standard solution mixture (AOZ, AMOZ, AHD, SEM and DNSAH) (Fig.1).

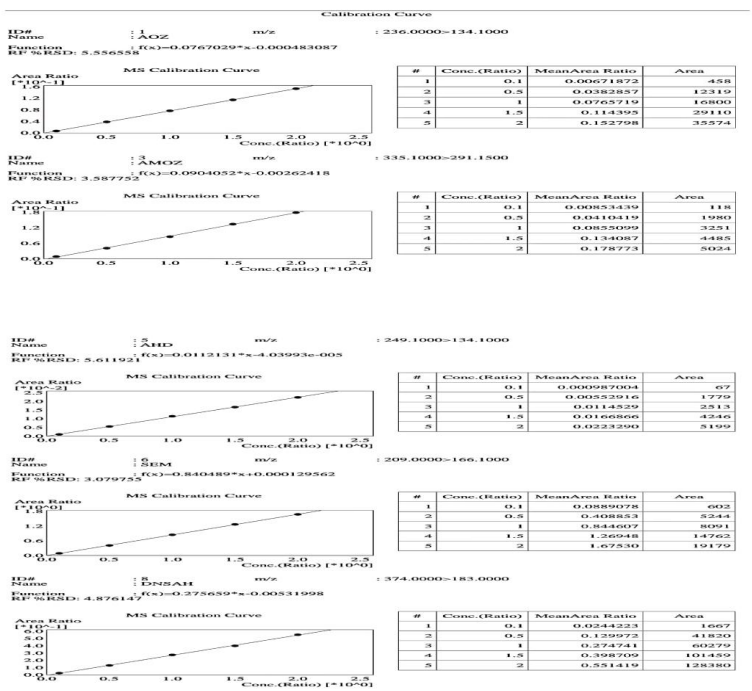



**Figure 1.** Calibration curve.

**Results and Discussion**

According to REGULATION (EU) NO. 37/2010 of the Comission of December 22.2009 regarding pharmacologically active substances and their classification according to the maximum residual limits in food products of animal origin, nitrofurans including furazolidone are included in table 2 of the Annex where the prohibited substances are mentioned, for which the maximum allowed limit cannot be established.

To determine the amount of residues of substances for which a limit is not established, the method was validated in the laboratory and the limits of detection and quantification were established through tests. The detection limit was determined as the arithmetic mean of the analyte concentration plus three times the standard deviation, and the quantification limit as the mean of the analyte concentration plus ten times the standard deviation. The Decision Limit (CCα) and the detection capacity (CCβ) were also calculated. The given parameters allow us to demonstrate what the residue content is and if it is necessary to receive a decision regarding the product with the given quantity of the substance.

**Table 1. Value calculated LOD and LOQ.**

| *Nr.* | *Compound* | *Matrix* | *MRL/MRPL* | *LOD* | *LOQ* |
|---|---|---|---|---|---|
| *1* | *AOZ* | *Muscle* | *0.5ppb* | *0.135 ppb* | *0.2ppb* |
| *2* | *AOZ* | *Egg* | *0.5ppb* | *0.133 ppb* | *0.2ppb* |
| *3* | *AMOZ* | *Muscle* | *0.5ppb* | *0.246 ppb* | *0.4ppb* |
| *4* | *AMOZ* | *Egg* | *0.5ppb* | *0.198 ppb* | *0.3ppb* |
| *5* | *AHD* | *Muscle* | *0.5ppb* | *0.196 ppb* | *0.3ppb* |
| *6* | *AHD* | *Egg* | *0.5ppb* | *0.201 ppb* | *0.3ppb* |
| *7* | *SEM* | *Muscle* | *0.5ppb* | *0.376 ppb* | *0.6ppb* |
| *8* | *SEM* | *Egg* | *0.5ppb* | *0.350 ppb* | *0.5ppb* |
| *9* | *DNSAH* | *Muscle* | *0.5ppb* | *0.284 ppb* | *0.6ppb* |
| *10* | *DNSAH* | *Egg* | *0.5ppb* | *0.263 ppb* | *0.6ppb* |

MRL/MRPL established by Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results, as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC in all metabolites it is 0.5ppb. After validating the ELISA immunoenzymatic screening method performed on the kits from R-Biopharm LOD values are between 0.13ppb and 0.37 ppb, the LOQ values are between 0.2ppb and 0.6ppb. These data allow us to draw a conclusion regarding the results obtained when examining the samples. (Tab. nr.2, nr.3).

**Table 2. Concentration of nitrofurans in egg samples.**

| Nr. | AOZ/conc. µg/kg | AMOZ/conc. µg/kg | AHD/conc. µg/kg | SEM/conc. µg/kg | DNSAH/conc. µg/kg |
|---|---|---|---|---|---|
| 1 | 0,32 | 0,20 | 0,07 | 0,01 | 0,02 |
| 2 | 0,13 | 0,18 | 0,24 | 0,14 | 0,02 |
| 3 | 0,25 | 0,08 | 0,01 | 0,30 | 0,03 |
| 4 | 0,16 | 0,13 | 1,01 | 0,06 | 0,02 |
| 5 | 0,17 | 0,15 | 0,17 | 0,28 | 0,02 |
| 6 | 0,14 | 0,11 | 0,03 | 0,18 | 0,03 |
| 7 | 0,16 | 0,14 | 0,06 | 0,24 | 0,01 |
| 8 | 0,29 | 0,07 | 1,02 | 0,17 | 0,03 |

**Table 3. Concentration of nitrofurans in muscle samples.**

| Nr. | AOZ/conc. µg/kg | AMOZ/conc. µg/kg | AHD/conc. µg/kg | SEM/conc. µg/kg | DNSAH/conc. µg/kg |
|---|---|---|---|---|---|
| 1 | 0,06 | 0,24 | 0,07 | 0,02 | 0,01 |
| 2 | 0,01 | 0,21 | 0,21 | 0,06 | 0,01 |
| 3 | 0,03 | 0,05 | 0,01 | 0,03 | 0,03 |
| 4 | 0,08 | 0,08 | 0,02 | 0,06 | 0,02 |
| 5 | 0,07 | 0,04 | 0,11 | 0,07 | 0,01 |
| 6 | 0,10 | 0,06 | 0,06 | 0,12 | 0,03 |
| 7 | 0,10 | 0,14 | 0,05 | 0,09 | 0,01 |

Of all the meat samples examined, no sample has a metabolite content higher than the LOD and LOQ. Egg samples no. 1, no. 3 and no. 8 have a higher content of AOZ, sample no. 4 and no. 8 a higher content of AHD, sample no. 3, no. 5 and no. 7 more SEM content.

**Conclusions**

Examining meat and egg samples allows us to draw conclusions on the use of substances from the nitrofuran group. So, the meat samples are free of residues of these substances. In some egg samples, contents of AOZ, AHD and SEM metabolites higher than the Limit of Quantification but lower than the MRL/MRPL.

**References:**

1. Merve Bacanlı, Nurşen Başaran, Importance of antibiotic residues in animal food, Food. Chem. Toxicol. *2019, Mar;125:462-466*. doi: 10.1016/j.fct.2019.01.033.
2. K. M. Cooper, R. J. Mccracken, M. Buurman, & D. G. Kennedy, Residues of nitrofuran antibiotic parent compounds and metabolites in eyes of broiler chickens, Food Additives and Contaminants, May 2008; 25(5): 548–556
3. Jane Kelly Finzi, Jose Luiz Donato, Mauro Sucupira, Gilberto De Nucci, Determination
of nitrofuran metabolites in poultry muscle and eggs by liquid chromatography-tandem mass spectrometry, Journal of Chromatography B, 824 (2005) 30–35 , doi:10.1016/j.jchromb 2005.05.01
4. R.J. McCracken and D.G. Kennedy, The detection, accumulation and distribution of The detection, accumulation and distribution of nitrofuran residues in egg yolk, albumen and shell, Food Additives and Contaminants, 14-Aug-2006
5. Noelia M.Valera-Tarifa, Patricia Plaza-Bolaños, RobertoRomero-González, José L.Martínez-Vidal, AntoniaGarrido-Frenich, Determination of nitrofuran metabolites in seafood by ultra high performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry, Journal of Food Composition and Analysis Volume 30, Issue 2, June 2013, Pages 86-93
6. SijieLiu, LeinaDou, XiaolinYao, WentaoZhang, BingxinZhao, ZonghanWang, YanweiJi, JingSun, BaochengXu, DaohongZhang, JianlongWang, Polydopamine nanospheres as high-affinity signal tag towards lateral flow immunoassay for sensitive furazolidone detection, Food Chemistry, Volume 315, 15 June 2020, 126310
7.Eric Verdon, Pierrick Couedor, Pascal Sanders, Multi-residue monitoring for the simultaneous determination of five nitrofurans (furazolidone, furaltadone, nitrofurazone, itrofurantoine,
nifursol) in poultry muscle tissue through the detection of their five major metabolites (AOZ, AMOZ, SEM, AHD, DNSAH) by liquid chromatography coupled to electrospray tandem mass spectrometry — In-house validation in line with Commission Decision 657/2002/EC,
Analytica Chimica Acta 586 (2007) 336–347 2007 doi:10.1016/j.aca.2007.01.024
8.Xiao Dong Yan , Xiu Zhi Hu , Hui Cai Zhang , Jing Liu & Jian Ping Wang Xiao Dong Yan , Xiu Zhi Hu , Hui Cai Zhang , Jing Liu & Jian Ping Wang, Direct determination of furaltadone metabolite, 3-amino-5-morpholinomethyl - 2-oxazolidinone, in meats by a simple immunoassay, Food and Agricultural Immunology, Vol. 23, No. 3, September 2012, 203-215, DOI: 10.1080/09540105.2011.615060.
9.Regulation (EU) NO. 37/2010 of the Comission of December 22.2009 regarding pharmacologically active substances and their classification according to the maximum residual limits in food products of animal origin. (access
10.Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results, as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC.

**THE PROSPECTS OF APPLICATION OF AMINOPROTEIC EXTRACTS FROM YEASTS OF WINE SEDIMENTS**

Efremova N., Besliu A., Chiselita N., Chiselita O., Tofan E., Danilis M.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: efremova.nadejda@gmail.com

CZU:663.26:579.67 https://doi.org/10.52757/imb22.55

Waste recycling contributes to obtaining of valuable products needed for a large number of economical directions. Over the last decade, the intensive study of reutilization of wastes is important. Yeasts from wine production can serve as prime source for food and feed additives with high biological value. Waste of wine production can be used for the production of natural cosmetics.

Taking into consideration the above, the aim of the research was to obtain aminoproteic extracts from the biomass of yeasts from the waste of the wine industry for use in the animal husbandry sector. In the research, the sediments from the production of dry white Rkatsiteli, red Merlot and Cabernet wines, offered by the wine complex «Cricova» SA and taken after the fermentation of the wine, were used.

For this were used some procedures of autolysis such as utilization of glacial acetic acid, homogenization and utilization of phosphate buffer. Depending on the method of autolysis chosen for the study, the following indicators of the biochemical composition of yeast from the obtained extracts were obtained. Thus, the protein content varied within 45.0-67.5% dry weight, carbohydrates 4.3-8.0% dry weight, catalase activity from 709.3 to 1436.9 mmol/min/mg protein, SOD activity – from 6.2 to 44.5 U/mg protein.

The development of technologies for the production of feed protein and other biologically active substances, based on waste from the wine industry, is relevant, both in terms of the safe use of this raw material and the elimination of environmental risks. Disposal of production waste that pollutes the human environment is one of the most important environmental and economic problems of society. A lot of waste is generated during the production of wine. Complex processing of secondary raw materials of winemaking is recognized not only as necessary and useful from the point of view of environmental protection and recreational activities, as it helps to reduce environmental pollution, but also as a highly efficient type of commercial activity. The use of yeast as a source of bioactive substances and complexes is one of the important areas of modern biotechnology.

*The results were obtained in the framework of the project 20.80009.5107.16. "New biologically active microbial preparations for increasing the reproductive and productive potential of animals of zootechnical interest", financed by NARD.*

# THE EFFECTS OF RATION MEDICATION WITH ZooBioR ON SOME PARAMETERS OF MINERAL METABOLISM IN YOUNG CHICKEN

Macari V., Pistol Gh., Gudumac V.[1], Rotaru A., Putin V., Rotaru L., Pavlicenco N., Pantea V.[1], Chișlari Iu.
*Technical University of Moldova, Chisinau, Republic of Moldova*
[1]*State University of Medicine and Pharmacy N. Testemițanu, Chisinau, Republic of Moldova*
e-mail:macvasile@mail.ru

CZU:636.087.8:636.52/.58 https://doi.org/10.52757/imb22.56

Birds represent the category of animals with an intense metabolism and a rapid transition of food through the digestive tract, technological stress being also present in the poultry industry. Thus, the exploitation of animals provides for the provision of impeccable microclimate, accommodation and food conditions, which, however, do not exclude the negative impact of technological stress on health, productivity, and quality of products. The role of medicinal remedies is great in the complex process of counteracting the undesirable consequences of the impact of technological stress.

Of great interest is the study of medicinal products with anti-stress, adaptive, and growth-stimulating actions, with positive impact on the health and productive potential of animals. In this context, the medicinal product ZooBioR, the object of our study, is of particular interest. It was successively extracted, using advanced technologies, from the biomass of the cyanobacterium Spirulina platensis. The research problem, carried out in the study, managed to take a step forward in the trend of the last decades in veterinary pharmaceuticals as to move from synthetic to natural pharmaceutical products.

The study was conducted on young, healthy laying hens (n=70), divided equally into five groups: one control and 4 experimental. The birds included in the research were analogous in terms of age, physiological state, origin, body weight, and operated under identical conditions. The ration of the birds in the control group was intact, and in 4 experimental groups (EL) it was supplemented with the ZooBioR product, in different doses (5.0; 10.0; 15.0 and 20 mg active substance/kg feed), product which was administered during the study. In order to assess the biological activity of the tested product, the state of health, at the beginning of the study, and subsequently during the study, including at the end of the experiment, the birds were examined, and in 5 hens from each group, the body temperature and respiratory movements per minute were determined. For laboratory research, blood samples were taken in three stages: at the beginning of the study, until the birds' feed was supplemented with the ZooBioR remedy, from 5 random hens; during the study, from 5 birds from each group - approximately 1 month after the start of the study, as well as later, at the end of this study, which coincided with the 129th day of research. It was established that the ZooBioR product taken into study was well tolerated by young chickens during the entire experimental period (129 days), a fact also confirmed by means of the body temperature and respiratory movements. In addition, these clinical parameters were more favorable in hens from the experimental groups, which benefited from the researched product, values that allow us to think of adaptive and anti-stressor properties of this ecological product. The biochemical results highlighted the positive impact of the natural remedy ZooBioR on the mineral metabolism in hens, in the first technological phase of laying eggs, both by the increase at the end of the study of Ca in the serum by about 6-10% and the decrease in three EL of P by 12-26 % respectively compared to the control, trends also confirmed by the Ca/P ratio, which in the 1st investigation was lower, and in the 2nd investigation, on the contrary, higher compared to the control. At the end of the study, Fe in EL 1 and 2 (low and minimal doses) had an increasing trend, and in EL 3 and 4 (high and maximum doses) a decreasing trend compared to the control.

The product ZooBioR, administered to hens in different doses, is well tolerated and has a beneficial influence on mineral metabolism, a fact confirmed both by the evolution of body mass and egg production.

**STABILITY OF SOME VEGETABLE OILS SUPPLEMENTED WITH ASTAXANTHIN AS ANTIOXIDANT**

Plingau E., Rudi L., Miscu V., Iatsko I.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: plingau_ecaterina22@gmail.com

CZU:665.3 https://doi.org/10.52757/imb22.57

Microalgae-derived antioxidants are starting to gain popularity in the processing of functional and organic foods. Astaxanthin (AXT) is included in the list of these antioxidants. The antioxidant potential of AXT allows food technologists to offer a wide range of functional foods. Incorporating astaxanthin into oils is a promising alternative to using this pigment. Vegetable oil can be one of the main factor in the solubilization and stability of astaxanthin, as well as its bioavailability, thus determining the fields of application of the final product. Research has been conducted to establish the antioxidant effect of natural astaxanthin from biomass of green microalga *Haematococcus pluvialis*, which was added to oils. The method of inducing thermal oxidation at a temperature of $60^0$C was used with the monitoring of the formation of conjugated dienes at 234 nm in a mixture of oil/hexane at a dilution of 600 times. Astaxanthin from *H. pluvialis* biomass was supplemented to vegetable oils at the concentration of 0.26 mg/ml. Oils of sunflower, olive, sesame, almond, poppy seeds and walnut kernels were used.

The conditions of thermal oxidation of vegetable oils have highlighted the stability of oils with a high content of oleic acid. Thus, during 180 min in olive oil, the content of conjugated dienes increased by 1.7 times. In sesame seed oil and almond oil, the content of conjugated dienes increased by 2.2 times. The highest values of the content of conjugated dienes were found in walnut and sunflower oils, the increase was 4.7 and 4.3 times, respectively. In poppy seed oil, the content of conjugated dienes increased by 3.7 times. The difference in the rate of development of oxidative processes in oils has been established. In poppy seed oil, the oxidative process started quickly from the first 30 min of exposure to a temperature of 60°C. In olive oil, high levels of conjugated dienes were recorded after 120 min of hyperthermia. The most stable oils to the process of thermal oxidation were olive, almond and sesame oils, which have a high content of oleic acid. Poppy seed oil, walnut oil and sunflower oil recorded the highest absorption values at 234 nm.

Thermal oxidation process in the case of walnut oil was determined to be the most aggressive, while the content of conjugated dienes increased throughout the entire period of the experiment.

Natural astaxanthin in the composition of vegetable oils slowed down the process of thermal oxidation of oils, which in the case of olive oil was determined after 180 min at a temperature of 60°C. The content of conjugated dienes in olive oil with AXT increased by 32% or 1.3 times compared to native oil under identical conditions. The content of conjugated dienes in sesame, almond and walnut oils was significantly reduced by 30-34% with the addition of AXT. The strong antioxidant effect of astaxanthin was found in the case of poppy seed oil, for which the formation of conjugated dienes was reduced by 42%. In this case, the oxidative process was noticeably delayed by 60 minutes when exposed to high temperatures compared to native oil subjected to the same experimental conditions. In the case of sunflower oil, which had a high percentage of thermal oxidation, the addition of AXT reduced the formation of conjugated dienes by 22% during the course of the experiment.

Natural astaxanthin, being a microalgae-derived bioactive compound, showed protective antioxidant properties for vegetable oils subjected to oxidation at high temperatures. This effect was more pronounced in oils with a high content of oleic acid. In the case of polyenoic vegetable oils containing predominantly C 18:2 and C 18:3 fatty acids, lower absorbance values characteristic of conjugated dienes indicated the presence of the antioxidant effect of AXT, which was manifested in the ability to annihilate free radicals.

The study revealed the antioxidant potential of AXT and its ability to stop the oxidation of vegetable oils under high temperature conditions. In conclusion, astaxanthin is a potent antioxidant for thermal protection of vegetable oils.

# USING THE PEARSON CORRELATION BETWEEN PH, TDS AND ORP PARAMETERS WITH MALON DIALDEHYDE AS THE BIOCHEMICAL INDICATOR OF THE PORK QUALITY

Stici V., Burlacu S., Enciu V., Racul A.
*State Agrarian University of Moldova, Republic of Moldova*
e-mail: sticiv.uasm@gmail.com

CZU:636.4.033 https://doi.org/10.52757/imb22.58

The aim of the paper is to compare the trend of quality indicators in pork meat traceability by defining the biochemical mechanism of lipid oxidation. In the pork samples holded at three temperature levels (low - 3$^0$C, medium - 15$^0$C and high - 30$^0$C) over a period of 96 hours, the measurements were performed using UV-VIS spectroscopy for the chromogen concentration (at $\lambda$ = 532 nm) that is obtained as the result in the biochemical reaction with the thiobarbituric acid (TBA). At the same time physico-chemical parameters of the acidity pH, the conductivity TDS and the oxidation-reduction potential ORP were evaluated in these samples to calculate the Pearson correlation as a fundamental criterion in arguing the malondialdehyde as a marker of quality in the biochemical mechanism of pork alteration. The comparative analysis of the trend in the evolution of the used indicators represents the methodological support for the implementation of the new high-performance technologies in the food safety of meat products.

**Keywords**: pork meat, malondialdehyde, thiobarbituric acid, acidity, conductivity, oxidation-reduction potential

**USE OF THE EXTRACTS OF SPIRULINA BIOMASS CONTAINING PHYCOCYANIN**

Zosim L., Bulimaga V., Trofim A., Elenciuc D.
*State University of Moldova, SRL Phycobiotechnology*
e-mail: zosim_liliana@yahoo.fr

CZU:573.6.086:582.232.2 https://doi.org/10.52757/imb22.59

In recent years, the attention of researchers is increasingly directed towards the exploration of natural antioxidants, including those obtained from cyanobacteria and microalgae. Some studies have shown that spirulina as dietary supplement and various extracts obtained from spirulina biomass can inhibit some forms of cancer in humans and animals [1], as well show antiviral, antibacterial and anti-inflammatory actions. A valuable component of the aqueous extracts obtained from spirulina are phycobiliproteins, especially phycocyanin. The antioxidant action of phycocyanin on hydroxyl and peroxyl radicals is due to the presence of conjugated bonds in phycocyanobilin [2]. The contribution of some amino acid residues from the polypeptide chain to this process cannot be excluded [3]. The aim of this work was: obtaining phycocyanin preparations from spirulina biomass enriched with zinc and determining their antioxidant activity.

Materials and methods. In order to obtain the spirulina biomass used as a source of phycocyanin, the cyanobacterium *Spirulina platensis* CNM-CB 02 was cultivated on the modified Zarrouk medium for 10 days, respecting the optimal cultivation parameters. The spirulina biomass enriched with zinc, as well and phycocyanin was obtained by cultivating spirulina under the same conditions, but with the supplementation of zinc acetate (20mg/l) in the growing medium. The antioxidant activity of the extracts was determined by the ABTS+ cationic radical decolorization method [4].

To determine the extractant that ensures a higher yield and purity of phycocyanin in the extract, three variants of extracts were performed: aqueous extract, aqueous extract containing 1% $CaCl_2$ and10% ethanol containing 1% $CaCl_2$. The extractant that provided the highest yield and purity of phycocyanin was found to be the solution of 1% $CaCl_2$ in 10% ethanol. The comparative study of the antioxidant capacity of the researched phycocyanin extracts highlighted higher values of the degree of inhibition in the case of extracts obtained from zinc-enriched biomass that vary within the limits of -19.0-26.60%, compared to the values evaluated for the extracts with phycocyanin content extracted from biomass grown under standard conditions (13.60-18.53%).

Thus, all phycocyanin preparations obtained from spirulina biomass show significant antioxidant capacity when determined by the reaction with ABTS+, % inhibition prevailing in the phycocyanin samples obtained from zinc-enriched biomass compared to the preparations containing phycocyanin obtained from standard biomass.

**References:**

1. Czerwonka A., Rzeski, W. Anticancer effect of the wat extract of a commercial *Spirulina (Arthrospira platensis*) product on the lung cancer A549 cell line. Biomedicine and pharmacotherapy, 2018, Vol.106, p.292-302
2. Anamika Patel, Sandhya Mishra, P K Ghosh. Antioxidant potential of C-phycocyanin isolated from cyanobacterial species. *Lyngbya*, *Phormidium* and *Spirulina spp*. Indian J. of Biochem and Biophysics.Vol.43, N2 ,2006, p.25-31.
3. Romey et al. 2003; C-phycocyanin: A biliprotein with antioxidant, anti-inflamatory and neuroprotective effects. Current protein and peptide science, 2003, 4, p.207-216;
4. Re R., et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. In: Free Radical Biology & Medicine, 1999, v.10, p.1231-1237.

*The given research was carried out within the project "Determination of Bioactivity and Antimyeloma Properties of Various Cyanobacteria", project number- 22.80013.5107.2TR*

RED

# Biotechnology

Health, Medical, Diagnostics

**TRANSMISSIBLE PARASITIC ZOONOSES OF THE SPECIES *APODEMUS URALENSIS* (Pallas, 1771)**

Chihai O. [1], Nistreanu V. [1], Larion A. [1], Talambuta N. [2], Rusu S.[1], Zamornea M. [1], Melnic G. [1], Kolodrevski O. [3]
[1]*Institute of Zoology, Republic of Moldova*
[2]*Free International University of Moldova, Republic of Moldova*
[3]*Theoretical High School Public Institution „Alecu Russo", Orhei, Republic of Moldova*
e-mail: olegchihai@yahoo.com

CZU:574.3:599.323.4 https://doi.org/10.52757/imb22.60

Zoonoses are contagious diseases caused by various pathogens (prions, viruses, bacteria, fungi, protozoa, helminths, arachnids, insects), common to humans and animals, and represent a major public health problem.

Pygmy field mouse inhabits the forest edge and open-type biotopes: meadows, grasslands, agrocoenoses, fallow ground. It is a species with a lower frequency compared to other small rodents and has accessorial ecological significance.

The aim of the study was to describe the epidemiological characteristic of the parasite fauna in *Apodemus uralensis* (Pallas, 1771).

The research was conducted in the laboratory of Parasitology and Helminthology of the Institute of Zoology, on *A. uralensis* captured from various biotopes of the Republic of Moldova. Laboratory parasitological investigations were performed by total dissection of previously euthanized rodents, with microscopic examination of the muscles (masseter muscles, arms, diaphragm) and internal organs (trachea, lungs, heart, tongue, esophagus, stomach, small intestine, large intestine, liver, spleen, kidney, bladder) in order to establish the structure of the helminthofauna and to determine the parasitological indices. The identification of the species was performedaccording to morphological criterion according to standard methods.

The taxonomic structure of the identified parasitic species falls into 3 classes, 7 orders, 13 families, 14 genera and 15 species.

There are 3 species (*Syphacia stroma, Syphacia obvelata, Strongyloides ratti*) in the category of parasitoses with zoonotic impact (parasitozoonoses), in the one with mixed impact (zoonotic + epizootic) there are 3 species (*Hydatigera taeniaformis larvae, Capillaria hepatica*), and in the category of rodent invasions there are 7 species (*Paranoplocephala omphaloides, Catenotaenia cricetorum, Skrjabinotaenia lobata*, *Rodentolipis straminea*, *Heligmosomoides polygirus, Mastophorus muris*, *Trichuris muris*).

The nosological characteristics of helminthoses includes 2 categories: Cestodoses teniosis - 10.0%, mesocestoidosis, paranoplocephalosis - 10.0%, catenoteniosis - 5.0%, cryabinoteniosis - 10.0%, hydatigeriosis - 10.0%); Nematodes (syphacioosis - 20.0%, strongyloidosis - 15.0%, capillaries -15.0%, heligmosomiasis - 5.0%, trichurosis - 15.0%, rodentolepiosis-5.0, mastophoresis - 20.0%). The epidemiological feature includes 3 categories of parasitic species.

The small rodents parasitofauna monitoring in different areas has a biomedical and epidemiological importance, in order to prevent the transmission of invasive forms to humans and other mammals involved in the biological cycles of parasites with zoonotic and epizootic role. Therefore, there are necessary measures to decrease the level of infestation in wild animals.

*The studies were performed within the State Program projects: 20.80009.7007.12 „Diversity of hematophagous arthropods, zoo- and phytohelminths, vulnerability, strategies for tolerating climatic factors and elaboration of innovative procedures for integrated control of species of socio-economic interest" and20.80009.7007.02 "Evolutive changes of economically important terrestrial fauna, of rare and protected species in the conditions of anthropic and climatic modifications".*

# PATHOGENIC AGENTS OF ACUTE DIARRHEA DISEASE FROM THE ENTEROBACTERIACAEA FAMILY AND THEIR ANTIBIOTIC RESISTANCE

Gutu N.
*MTA Buiucani Public Health Medical Institution, Chisinau, Moldova*
*Doctoral school of biological, geonomic, chemical and technological sciences*
e-mail: nadejda_gutu@mail.ru

CZU:579.61:[579.21+578.2+614.2] https://doi.org/10.52757/imb22.61

The Enterobacteriaceae family includes a large number of bacteria that inhabit the intestines of humans and animals, being spread with fecal matter everywhere in the environment. The representatives of the family are characterized by resistance to antibiotics, especially beta-lactam ones (penicillins, cephalosporins, cephamycins and carbapenems), a fact determined by the production of beta-lactamases by these microorganisms.

The aim of this study was to identify the pathogens from the Enterobacteriaceae family, which caused acute diarrheal diseases (ADD) in patients who addressed the AMT Buiucani Public Health Medical Institution during the years 2020-2021, and to highlight the spectrum of antibiotic resistance of isolated specimens.

934 clinical samples (feces) were collected from patients and investigated in the microbiological laboratory of the mentioned institution. Thus, from the positive samples (424) there were isolated and identified pathogenic bacteria (*Shigella spp*., *Salmonella spp*.) and conditionally pathogenic bacteria (*Klebsiella spp.*, *Proteus spp*., *Citrobacter spp*., *Enterobacter spp*., *Seratia*, *Providence*, *Morganella morganii*, *Pseudomonas aeruginosa*) from the Enterobacteriaceae family, which can cause food poisoning and acute diarrheal disease. The sensitivity to antibiotics of the isolated microorganisms was determined by the diffusimetric method with the use of antibiotic sets specific to each pathogen, selected according to EUCAST recommendations and national protocols. The spectrum of resistance of the identified agents is shown in the figure.

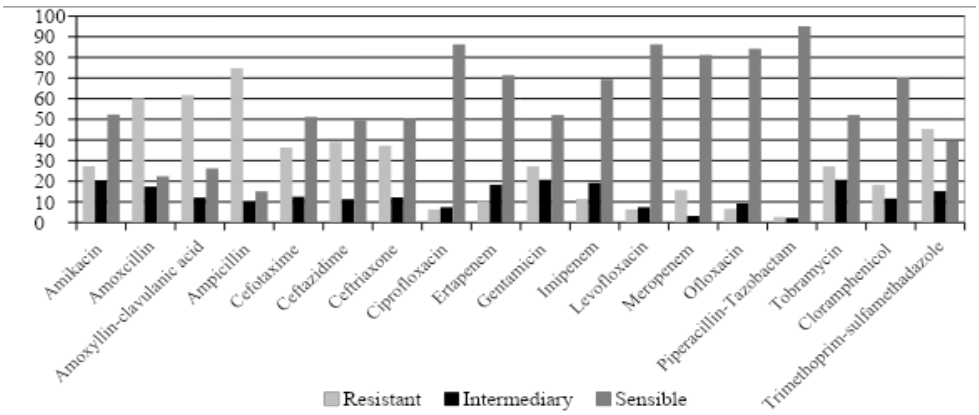



**Figure 1**. Spectrum of antibiotic resistance of ADD agents

Pathogenic and conditionally pathogenic enterobacteria show a more pronounced sensitivity to such antibiotics as Ciprofloxacin, Levofloxacin and Ofloxacin and increased resistance to Amoxicillin, Ampicillin and Amoxylin – clavulanic acid. As can be seen, cephalosporins become antibiotic-resistant, then aminoglycosides and ampicillins. Resistant bacteria proliferate by natural selection when those sensitive to antimicrobial preparations are removed by antibiotics.

*The study was performed within the doctoral project "Pathogens of acute diarrheal diseases - morpho-cultural features, methods of identification, antibiotic resistance and the dynamics of spread in Chisinau city."*

# ANTIMICROBIAL ACTIVITY OF POLYSACCHARIDES SOME BACTERIA GENUS ENTEROCOCCUS AGAINST PNEUMONIA PATHOGENS

Israyelyan A.[2], Tkhruni F.[1], Balabekyan T.[1], Beglaryan L.[2], Aleqsanyan L[2], Farsiyan A.[3]
[1]*Probiotics Production Sector, Scientific and Production Center "Armbiotechnology" NSPO, National Academy of Science, Armenia*
[2]*Laboratory of Microbiology, Artsakh Scientific Center State Non-Commercial Organization (SNCO)Stepanhakert, Armenia*
[3] *Yerevan Medical State University, Armenia*
e-mail: arevik_israelyan@mail.ru

CZU:579.61:616.24:577.114.4 https://doi.org/10.52757/imb22.62

Previously, protein-like fractions with antimicrobial activity (AMP) were obtained using the gel (Sephadex G-25) filtration method for the purification of cultural liquid (CL) of Enterococcus strains isolated from fermented milk of donkeys. The antimicrobial activity of strains of the genus Enterococcus is due to the synthesis of law weight protein-like substances and depends on time, temperature and the composition of the growing medium. The greatest antimicrobial activity of the studied strains takes place at a growing temperature (42°C).

After cultivation of the investigated LABs were isolated and purified polysaccharides from CL. Comparison of the results showed the difference in the effectiveness of the influence of polysaccharides depends on the pathogens source, the polysaccharide concentration, and the generic nature of the strain causing pneumonia. From investigated 21 strains 8 had polysacharides, but only 2 have antimicrobial activity. These polysaccharides inhibited the growth of the pathogenic bacteria isolated from people with covid.

The aim of this work was to study some LAB strains of the Enterococcus genus with probiotic properties synthesizing polysaccharides on the ability to inhibit the growth of pathogenic bacteria that cause pneumonia.

**Figure 1.** Antimicrobial activity of polysaccharides and metabolites some bacteria genus *Enterococcus* against *St. pneumonia* fl-510(isolated from throat)

The HPLC method used showed that the isolated polysaccharides of Enterococcus strains with antimicrobial activity consist of glucose and galactose molecules. Now are studying the physicochemical properties of polysaccharides, which can be promising in future. It is concluded that the use of metabolites of the genus Enterococcus as bioinhibitors is promising.

*Acknowledgments: This work realized with financial support of the RA MES State Committee of Science project "21AA-005, 2021*

# EVALUATION OF THE FUNCTIONAL ACTIVITY OF T LYMPHOCYTES, CONCENTRATION OF IL-4, IL-10 and IFN-γ AT THE EXPOSURE TO BIOACTIVE COMPOUNDS EXTRACTED FROM *SPIRULINA PLATENSIS* IN PULMONARY TUBERCULOSIS

Lesnic E. [1], Privalova E.[2], Ghinda S. [2], Chiriac T. [3], Rudic V. [3]
[1] – *Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova*
[2] – *Institute of Phthysiopneumologie „Ch. Draganiuc", Republic of Moldova*
[3] – *Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: evelina.lesnic@usmf.md
CZU:615.322:582.232.2+616.24-002.5-08 https://doi.org/10.52757/imb22.63

**Summary**

This study presents the results of the influence of bioactive compounds extracted from *Spirulina platensis* on the functional activity of T-lymphocytes through BTRL by PHA, concentration of IL-4, IL -10 and IFN-γ in patients with pulmonary tuberculosis. The experiments were realised *in vitro* and were tested sulfated polysiccharides (SPS), BioR and BioR$^{Zn}$. Results: In patients with allergic reactions to anti-tuberculous treatment BioR$^{Zn}$ decreased the BTRL by PHA, but SPS and BioR increased. In patients with toxic and toxic-allergic reactions SPS and BioR increased and BioR$^{Zn}$ decreased. In patients with toxic and toxic-allergic reactions the concentration of IFN-γ was higher than in those with allergic reactions but IL-4 was higher in patients with allergic and toxic-allergic reactions. The exposure of the T-lymphocytes to SPS statistically decreased the level of IFN–γ, increased IL-4 and IL-10. Conclusions: obtained data confirmed the utility of SPS, BioR and BioR$^{Z}$ as remedies for pathogenic correction of disturbances caused by adverse drag events developed during the anti-tuberculous treatment in patients with pulmonary tuberculosis.

**Key words:** sulfated polysaccharides from Spirulina, BioR and BioR$^{Zn}$, pulmonary tuberculosis, T-lymphocytes, IL-4, IL -10 and IFN-γ.

## Introduction

Adverse drug events (ADE) during the anti-tuberculous drugs (ATD) can occur up to 20% of patients with pulmonary tuberculosis (PTB) and the most common is the drug ATD toxicity [1]. Drug toxicity (DT) appears the most frequently during the treatment with injectable aminoglycosides, followed by isoniazid, rifampicin and pyrazinamide [2]. Allergic reactions due to ATD are diagnosed in 4-6% and can be mild to severe such life-threatening conditions [1]. The correct management of ADE consists in the identification of the causing ATD, individualisation of the treatment according to the patient's clinical tolerance and metabolic disorders and desenzitization [3]. Studies showed that DT is often conditioned by the immune disorders, which finally worsen the clinical evolution and determine unfavourable outcome [4]. Current medical prescribing practices does not allow the systematic evaluation of the patient's immune state, even if it is an essential method of the personalized medicine for the improvement of the compliance and outcome [3]. Some researches revealead the immune-modulatory and anti-inflammatory effects of bioactive compounds (BAC) extracted from the *cyanobacterium Spirulina (Athrospira) platensis*. Particularly valuable are sulfated polysaccharides (SPS) which are polyanionic complexes located on the external surface of cell membranes and in the extracellular space. SPS is mainly based on fructose, rhamnose, xylose, mannose, glucose and galactose, various isomeric forms and types of glycosidic bonds, and the three-dimensional structure [5]. SPS extracted from *S. platensis* exhibits antiviral, antioxidant, antifibrotic, anticoagulant, antitrombotic, hypolipemiant activity and immune modulating effects through the mechanism of constitution of the tissue barrier. *Sodium spirulan,* a SPS isolated from *S. platensis,* increases the production of endothelial proteoglicans, providing an efficient anti-thrombotic activity, stabilizes the lysosomal membranes and increase the immune defense [6]. Despite the recent advances in ficobiotechnology the effects of BAC isolated from *S. platensis* on the cell-mediated immunity indicators in patients with PTB were not established.

**The aim** of the study was to investigate the effects of BAC extracted from *S. platensis,* on the indices of the functional activity of T lymphocytes, concentration of IL-4, IL -10 and IFN-γ in patients diagnosed with PTB, which developed ADE with the scope to select the compound with the greatest evidence in the improvement of the immune disorders.

**Material and methods**

The conducted study was analytical, prospective and case-control. In the study were included 110 patients, who encountered the including criteria: age more than 18 years old, diagnosed with PTB infiltrative form, culture drug-susceptible confirmed TB and new case. All patients provided the signed informed consent. The patients were distributed in 3 groups according to the ADE: 1st group – 37 patients with allergic reaction (type I reaction mediated by IgE), 2nd group – 49 patients with toxic and allergic reactions (type I mediated by IgE and type II mediated by IgG and IgM) and 3rd group -25 patients with toxic reaction (type II, mediated by IgG and IgM). The experiments were conducted *in vitro* using the peripheral lymphocytes selected from the venous blood samples, which were collected, when the adverse drug effects were established, according to the principles of the biological standardization and conduct of experiments, approved by the Research Ethics Committee of "Nicolae Testemițanu" SMPhU, protocol No. 14 from 20/10/2017 and carried out according to the Helsinki declaration with modifications (Somerset West Amendment, 1996). To evaluate the functional activity of T cells, was used the index of blast transformation of lymphocytes reaction (BTRL) to polyclonal mitogens - phytohemagglutinin (PHA), the concentration of interleukins IL-4, IL-10 and IFN-γ before and after the exposure to the BAC: SPS, BioR and $BioR^Z$ obtained at the Institute of Microbiology and Biotechnology from the R. of Moldova.Statistical analysis was performed using the SPSS 23.0 program. To test for significant differences between the parameters of the compared groups, Fisher's exact test and non-parametric Student's t-test were performed. The threshold for statistical significance was $p<0.05$.

**Rezultats and discutions**

When distributing patients by sex, was established the predominance of men *vs.* women ($p<0,001$ in all groups) with the ratio 1,8 in the 1st group, 1,6 - 2nd group and 2,4 - 3rd group without statistical significance at the comparision of the groups. Analyzing the age, it was found that the majority of patients in all groups were between 18 and 44 years old, and statistically predomminated compared with the patients older 45 years ($p<0,001$ in all groups), without differences between the groups. As well the average age did not differ significantly between the groups. So, according to the distribution acoording to the sex and age, the goups were comparable.

Radiological features of etensive TB affecting more than 3 lung segments statistically predominated in the 3rd *vs.* 1st group ($p<0,001$) and unsignificantly *vs.* 2nd group. Destruction of the lung parenchima statistically predominated in the 3rd *vs.* 1st and 2nd groups ($p<0,001$ in both groups). Disseminative opacitities were identified in every third case from all groups. Microscopic positive for AFB were the majority of patients, due to including criteria in the research of the drug-susuceptible confirmed pulmonary TB. The duration of the hospitalisation for the anti-TB treatment was significantly longer in the 3rd *vs.* 1st and 2nd groups ($p<0,001$ in both groups (Table 1).

**Table 1. Distribution of patients by sex, age, radiological and microbiological characteristics**

| Indicators | 1st group | 2nd group | 3rd group |
|---|---|---|---|
| Men (abs. no., %) | 24 (64,9) ◊ | 30 (61,2) ◊ | 17 (70,8) ◊ |
| Women (abs. no., %) | 13 (35,1) | 19 (38,8) | 7 (29,2) |
| Age 18-44 years (abs. no., %) | 25 (76,6) ◊ | 34 (69,4) ◊ | 15 (60,1) ◊ |
| Age older than 45 year (abs. no., %) | 12 (24,4) | 15 (31,6) | 10 (39,9) |
| Average age (years) | 36,3±2,38 | 35,4±2,07 | 36,0±2,78 |
| Extensive TB (abs. no., %) | 21 (56,7) | 32 (65,4) | 18 (72,1) ○ |
| Lung destruction (abs. no., %) | 22 (59,5) | 32 (65,3) * | 21 (87,5) ○ |
| Dissemination (abs. no., %) | 11 (29,7) | 13 (26,5) | 8 (32,1) |
| AFB positive (abs. no., %) | 26 (70,3) | 40 (81,6) | 20 (83,3) |
| Total duration of the treatment (days) | 74,9±7,76 | 73,8±5,46 * | 89,5±10,91○ |

*Note: Statistical test used Fisher's exact test.*

*◊ - statistical difference between the rate of men compared with women, and of patients between 18-44 and older 45 years, in each group, respectively;*

*○ – statistical difference detween the indices from the 1st group and 3 rd group;*

** - statistical difference detween the indices from the 2nd group and 3 rd group;*

The rate of patients with previous allergic reactions was low and did not differ between the groups. Comparing the clinical signs attributed to ADE, was estbalished the statistical predominance of pruritus in

the 1st and 2nd groups *vs.* the 3rd group (p<0,01 in both groups), due to including criteria. Nausea and vomiting, increased size of the liver, statisticaly predominated in the 3rd *vs.* 1st and 2nd groups (p<0,001 in both groups), concomitantly the hepatomegaly was more frequently in the 3rd *vs.* 2nd group (p<0,01). Paresthesis and decreased visual acuity predominated in the 3rd *vs.* 1st and 2nd groups. Laboratory disorders such as anemia, elevated alanine transaminase (ALT) and aspartate aminotransferare (AST) statistically predominated in the 3rd *vs.* 1st and 2nd groups (p<0,001 respectively) (Table 2).

**Table 2. Clinical and laboratory peculiarities of ADE (abs. no., %)**

| Indicators | 1st group | 2nd group | 3rd group |
|---|---|---|---|
| Allergic anamnesis (abs. no., %) | 2 (5,4) | 2 (4,1) | 1 (4,2) |
| Papular pruritus (abs. no., %) | 5 (13,5) ● | 6 (12,2) ∗ | 0 |
| Erythematous pruritus (abs. no., %) | 9 (24,3) ● | 10 (20,4) ∗ | 0 |
| Paresthesis (abs. no., %) | 3 (8,1) | 3 (6,1) | 3 (12,5) |
| Nausea, vomiting (abs. no., %) | 0 | 6 (12,2) ● | 8 (33,3) ○ |
| Decreased visual acuity (abs. no., %) | 0 | 0 | 1 (4,2) |
| Increased liver size (abs. no., %) | 2 (5,5) ● | 15 (30,6) ∗ | 15 (62,5) ○ |
| Anemia (abs. no., %) | 1 (2,7) ● | 3 (6,1) | 5 (20,8) |
| Elevated ALT (abs. no., %) | 4 (10,8) ● | 10 (20,4) | 8 (33,3) |
| Elevated AST (abs. no., %) | 2 (5,4) ● | 9 (18,4) | 7 (29,2) |
| Positive Thymol turbidity test (abs. no., %) | 1 (2,7) | 2 (4,1) 83 | 1 (4,2) |

*Note: Statistical test used Fisher's exact test.*
● *– statistical difference detween the indices from the 1st group and 2nd group;*
○ *– statistical difference detween the indices from the 1st group and 3 rd group;*
∗ *- statistical difference detween the indices from the 2nd group and 3 rd group;*

The analysis of the complete blood count established a higher number of leucocytes in the 3rd *vs.* 1st group (p<0,01). When analysing the rate of each type of neutrophils, was determined the statistical predomination of segmented, non-segmented and of the cells with toxic granulations in the 3rd *vs.* 1st group (p<0,001 respectively). The rate of eosinophils and lymphocytes was statistically higher in the 1st group *vs.* 3rd group (p<0,001 respectively). Concomitantly the rate of neutrophils with toxic granulations and eosinophils was higher in the 3rd group *vs.* 2nd group and eosinophils in the 2nd *vs.*group 3rd (p<0,05 respectively). The analysis of the BTRL by PHA established a lower proliferative activity in the 3rd *vs.* 1st group (p<0,001) and *vs.* 2nd groups (p<0,01), (Table 3). So, the patients with PTB which developed allergic, toxic and mixed reactions to ATD presented different disorders. Degree of disturbances was higher in patients with toxic ADE compared with those with allergic events.

**Table 3. Complete blood count in patients with ADE**

| Indicators | 1st group | 2nd group | 3rd group |
|---|---|---|---|
| Leucocytes (x$10^9$/L) | 7,5±0,34● | 8,4±0,37 | 9,1±0,75 |
| Segmented neutrophils (%) | 53,7±1,50● | 62,3±1,39 | 64,7±1,90○ |
| Nonsegmented neutrophils (%) | 3,6±0,48● | 3,7±0,65 | 5,0±0,94○ |
| Neutrophils with toxic granulations, (abs. no. and %) | 0● | 1 (2,0±2,02)∗ | 5 (20,8±8,29) |
| Eosinophils (%) | 7,5±0,75● | 2,4±0,33∗ | 1,4±0,30○ |
| Lymphocytes (%) | 28,1±1,50● | 24,7±1,36 | 21,7±2,00 |
| BTRL by PHA (%) | 61,7±1,42● | 54,9±1,12∗ | 48,1±2,47 |

*Note: Statistical test used nonparametric T Student.*
● *– statistical difference detween the indices from the 1st group and 2nd group;*
○ *– statistical difference detween the indices from the 1st group and 3 rd group;*
∗ *- statistical difference detween the indices from the 2nd group and 3 rd group;*

The BTRL by PHA after the exposure to different BAC isolated from *S. platensis* showed different effects as following:

$BioR^{Zn}$ significantly decreased the BTRL of T lymphocytes in the 1st group, SPS and BioR unsignificantly increased.

SPS and BioR significantly increased the functional activity in the 2nd and 3rd groups, and BioR$^{Zn}$ slightly reduced in the same group.

Comparing the groups, the SPS increased more evident the BTRL in 1st *vs.* 2nd and *vs.* 3rd groups and less evident in the 2nd *vs.* the 3rd groups.

BioR significantly increased the BTRL in 1st *vs.* 2nd group, 1st *vs.* 3rd group, as well in the 2nd *vs.* the 3rd group.

BioR$^{Zn}$ significantly decreased the functional activity in the 1st *vs.* the 3rd group, and in the 2nd *vs.* the 3rd group (Table 4).

**Table 4. *In vitro* evaluation of the BTRL by PHA at the exposure to the BAC**

| Indicators | Exposure to SPS | | Exposure to BioR | | Exposure to BioR$^{Zn}$ | |
|---|---|---|---|---|---|---|
| 1st group before<br>after the exposure | 68,1±1,21<br>70,2±0,56 | | 65,6±1,11<br>67,5±0,64 | | 67,6±0,90<br>61,9±0,86● | |
| 2nd group before<br>after the exposure | 58,3±1,12<br>61,9±1,11● | | 59,9±1,04<br>63,4±0,86● | | 63,7±0,95<br>62,1±0,74 | |
| 3rd group before<br>after the exposure | 48,0±1,11<br>54,2±3,64● | | 52,9±2,35<br>58,4±2,20● | | 57,1±2,24<br>56,3±1,68 | |
| Before/after the exposure | before | after | before | after | before | after |
| 1st *vs.* 2nd group | <0,001 | <0,001 | <0,001 | <0,001 | <0,01 | >0,05 |
| 1st *vs.* 3rd group | <0,001 | <0,001 | <0,001 | <0,01 | <0,001 | <0,01 |
| 2nd *vs.* 3rd group | <0,01 | <0,05 | <0,01 | <0,05 | <0,01 | <0,01 |

The IFN–γ plays an essential antiproliferative role and interleukins IL-4 and IL-10 determine anti-inflammatory effects. When the ADE were detected the concentration of IFN–γ was statistically higher in the 3rd *vs.* 1st and 2nd grous. The concetration of IL-4 was statistically higher in the 1st *vs.* 2nd group, and achieved the statistical threshold at the comparision with 2nd group. The concetration of IL-10 did not statistically differ among all groups. The exposure SPS statistically decreased the concentration of IFN–γ from the initial one, statistically increased the concentration IL-4 and insignificantly increased IL-10. Comparing the groups, the IFN–γ decreased more evidently in the 1st vs. 2nd and 3rd groups, as well in the 2nd *vs.* 3rd groups. The concentration of IL-4 increased significantly in the 1st *vs.* 2nd and 3rd groups, and the IL-10 did not changed significantly (Table 5).

**Table 5. The effect of SPS on the concetration of cytokines**

| Indicators | IFN –γ ng/L | | IL-4 ng/L | | IL-10 pg/L | |
|---|---|---|---|---|---|---|
| 1st group before<br>after the exposure | 71,8±4,13<br>53,3±4,03● | | 8,1±0,80<br>14,0±1,32● | | 0,81±0,03<br>0,90±0,02 | |
| 2nd group before<br>after the exposure | 101,9±5,37<br>83,1±5,60● | | 7,0±0,60<br>9,6±0,90● | | 0,77±0,01<br>0,83±0,02 | |
| 3rd group before<br>after the exposure | 124,9±5,34<br>110,5±5,12● | | 5,5±0,53<br>7,1±0,59● | | 0,82±0,03<br>0,93±0,02 | |
| | before | after | before | after | before | after |
| 1st *vs.* 2nd group | <0,001 | <0,001 | >0,05 | <0,01 | >0,05 | >0,05 |
| 1st *vs.* 3rd group | <0,001 | <0,001 | <0,05 | >0,001 | >0,05 | >0,05 |
| 2nd *vs.* 3rd group | <0,01 | <0,01 | >0,05 | <0,05 | >0,05 | >0,05 |

Spearman's correlation coefficient established moderate negative correlation between the BTLR by PHA and IFN –γ in the 1st and 2nd groups, and strong negative in the 3rd group. So, the high levels of IFN–γ determined a reduced proliferation of lymphocytes. The concentrations of IL-4 and IL-10 positively correlated with BTLR by PHA, at moderate degree in the 3rd group, meaning high concentrations of anti-inflammatory interleukins determined intense proliferation of the lymphocytes (Table 6).

**Table 6. Correlation between the values of BTLR by PHA and cytokines**

| Indicators | IFN –γ<br>r | IL-4<br>r | IL-10<br>r |
|---|---|---|---|
| 1st group | **-0,51**[3] | 0,37[2] | 0,25[1] |
| 2nd group | **-0,58**[3] | 0,48[2] | 0,37[2] |
| 3rd group | **-0,71**[4] | **0,57**[3] | **0,58**[3] |

**Spearman's correlation coefficient was calculated to determine the strength of the correlation between the BTLR by PHA and immunological parameters. Interpretation on the Chaddock scale: 1 at r 0.1-0.29 - very weak correlation, 2 at r 0.3-0.49 weak correlation; 3 at r 0.5-0.69 average correlation; 4 at r 0.7 and higher—strong correlation.*

**Conclusions**

The effects of bioactive compounds SPS, BioR and BioR$^{Zn}$ were selective and differentiated. In patients with allergic reactions BioR$^{Zn}$ decreased the BTRL by PHA, and SPS and BioR increased. In patients with toxic and toxic-allergic reactions SPS and BioR increased and BioR$^{Zn}$ slightly reduced. In patients with toxic and toxic-allergic reactions the concentration of IFN-γ was higher than in those with allergic reactions, IL-4 was higher in patients with allergic and toxic-allergic reactions. Was established negative correlation between IFN–γ, and positive between IL-4, IL-10 and BTRL by PHA. The exposure of the T-lymphocytes to SPS statistically decreased the level of IFN–γ, increased IL-4 and IL-10, with higher evidence in patients with allergic reactions.

The results confirmed the utility of SPS, BioR and BioR$^{Z}$ as remedies for pathogenic correction of the immune disturbances caused by adverse drag events developed during the anti-tuberculous treatment in pulmonary tuberculosis.

**References:**

1. *WHO*: Guidelines for treatment of drug-susceptible tuberculosis and patient care, 2017. Disponibil : https://www.who.int/tb/publications/2017/dstb_guidance_2017/en/
2. Li Y, Zhu Y, Zhong Q, Zhang X, Shu M, Wan C. Serious Adverse Reactions from Anti-tuberculosis Drugs Among 599 Children Hospitalized for Tuberculosis. Pediatr Infect Dis J. 2017 Aug;36(8):720-725. doi: 10.1097/INF.0000000000001532. PMID: 28060046.
3. Ministerul Sănătății, Muncii și Protecției Sociale al Republicii Moldova. Protocol Clinic Național. Tuberculoza la Adult. PCN-123. Chișinău, 2020. 152 p. Available on: https://msmps.gov.md/wp-content/uploads/2021/02/PCN-123-Tuberculoza_la_adult.pdf.
4. Martino M., Lodi L., Galli L., Chiappini E. Immune Response to Mycobacterium tuberculosis: A Narrative Review. In: *Frontiers in Pediatrics*, Vol. 7, 2019, https://www.frontiersin.org/article/10.3389/fped.2019.00350
5. Rudi L., Chiriac T., Cepoi L. Factorii tehnologici și calitatea biomasei de spirulină. Chișinău, 2020, et. ,,Artpoligraf", 242 p.
6. Se-Kwon Kim, Yong-Xin Li. Medicinal Benefits of Sulfated Polysaccharides from Sea Vegetables. Ed. Advances in Food and Nutrition Research, Academic Press, Volume 64, 2011, Pages 391-402, ISSN 1043-4526, ISBN 9780123876690, https://doi.org/10.1016/B978-0-12-387669-0.00030-2.

**EFFICACY AND SAFETY OF THE VIDEO-OBSERVED TREATMENT IN PATIENTS WITH PULMONARY TUBERCULOSIS**

Malic A., Niguleanu A., Osipov T., Lesnic E.
*State Medicine and Pharmacy University Nicolae Testemitanu, Republic of Moldova*
e-mail: evelina.lesnic@usmf.md

CZU:616.24-002.5-08 https://doi.org/10.52757/imb22.64

Telemedicine for providing support of high-risk groups is now gaining-momentum worldwide. In this context, the European Commission elaborated the strategic approach requiring a long-term program for implementation of telemedicine. As a result, the Republic of Moldova (RM) did the first step for implementing the video-observed treatment (VOT) in the therapeutic management of tuberculosis (TB) patients. VOT is based on the principle when the staff involved in its performing can observe the administration of the antituberculous drugs using electronic devices (personal computer, notebook, smartphone with Android system) through a web camera. Patient can be treated by VOT if has an available electronic device, a web camera, broadband Internet is residing in the RM and can administrate independently the treatment. . Excluding criteria from VOT were deep social economic vulnerability, migration, homelessness, detention, mental disorders and harmful habits associated with psychic impairment, as well as severe comorbid condition.

The aim was to assess the efficacy and safety of the video-observed treatment (VOT) in patients with pulmonary TB.

A prospective case-control study included 114 patients with PTB treated with VOT, distributed in the group 1 (G1) - 26 cases treated in both therapeutic phases using the VOT and the group 2 (G2) - 88 cases treated by VOT only in the continuation phase in the period 2019-2022 in Chisinau was conducted.

Distribution according to sex: men/women rate was 1,4 in G1 vs 2,7 in G2, and the majority of patients from both groups were between 18 and 44 years 15 (58%) *vs* 61 (69%) cases, respectively. New cases were 24 (92,3%) *vs* 83 (94,3%) and previously treated for TB were 2 (7,6%) *vs* 5 (5,7%) patients, respectively. By the family doctors as symptomatic patients were detected 3 (11,5%) *vs* 14 (15,9%) and as high-risk groups 6 (23,1%) *vs* 7 (7,9%) cases, directly addressed to the institution offering the VOT were 7 (30,1%) *vs* 17 (19,3%). Positive results of the sputum smear for AFB were 4 (15,4%) *vs* 34 (38,6%), $p<0,01$ and culture positive were 5 (19,2%) *vs* 44 (60,1%) ($p<0,001$), GeneXpert MTB positive results were obtained in 5 (19,2%) *vs* 50 (56,8%), ($p<0,001$), and resistant to rifampicin were 2 (7,6%) *vs* 19 (21,6%), ($p<0,001$) respectively in both groups. All patients were diagnosed with pulmonary infiltrative TB, however bilateral involvement was determined in 5 (19,2%) *vs* 30 (34,1%) and lung destrucion in 3 (11,5%) *vs* 15 (17,1%) cases respectively. Therapeutic success was established in 24 (92%) *vs* 78 (89%) cases, died 1 (2%) patient as result of the comorbid state and 1 (1.3%) continued the treatment in G1 *vs* 10 (11%) in G2. Adverse drug reactions were minor, managed remotely and included only gastroientestinal disorders in 3 (11,3%) *vs* 2 (2,3%).

**Conclusions:** The VOT in patients with pulmonary TB, showed a high efficacity and safety, regadless the treatment phase during which was implemented. Disease related characteristics, such as case-type, clinical form and severity, positive microbiological state and multi-drug resistance din not show an evident impact on the outcome, as a consequence of excluding criteria. Even the rate of adverse drug events was low, one death was registered and was determined by other cause than the progession of tuberculosis. VOT can be implemented in the management of TB patients in actual epidemiological state of the Republic of Moldova, if a complex of supporting measures will be offered to the patients, as an electronic device with a web camera, broadband Interne and financial support during the treatment.

# THE IMPACT OF THE COORDINATIVE COMPOUNDS, THIOSEMICARBASIDE DERIVATES ON THE OXIDATIVE STRESS INDICES IN *EX VIVO* EXPERIMENTS

Pantea V., Lesnic E., Andronache L.
*Laboratory of biochemistry, Nicolae Testemitanu State University of Medicine and Pharmacy, Republic of Moldova*
e-mail: valeriana.pantea@usmf.md

CZU:577.3+57.085 https://doi.org/10.52757/imb22.65

Background: An increased interest is actually offered in the evaluation of the impact of the new copper coordinative compounds (CCC) - thiosemicarbaside derivates. CCC showed important antitumoral properties, however their influence on the oxidative stress (OS) has not been studied. The oxidative stress (OS) is caused by the imbalance between the systemic manifestation of the reactive oxygen species (ROS) and the biological system's ability to detoxify them.

Aim: to study the influence of the new copper coordinative compounds, thiosemicarbaside derivatives, coded as CMA-181, CMC-34, CMD-8, CMG-41 andCMJ-33 on the OS indices in *ex vivo* experiments.

Material and methods: the new CCC, thiosemicarbaside derivatives, coded as CMA-18, CMC-34, CMD-8, CMG-41, and CMJ-33 developed at the State University of Moldova in the Laboratory "Advanced materials in biopharmaceutical and technical" were evaluated in several *ex vivo* experiments in 2 concentrations – 1,0 and 10,0 mM/L, in which was used the peripheral blood samples collected from 8 conventionally healthy individuals [1, 2, 3]. The markers of OS were assessed the activity of malondialdehyde (MAD), superoxide dismutase (SOD) and catalase (CAT).

Results and discussions: Our research showed different changes exerted by the new CCC on the indices of the OS in *ex vivo* experiments in conventionally healthy individuals. The most pronounced increase of SOD compared to the initial level was registered for CMA-181 (10,0 mM/L) and (1,0mM/L), CMC-34 (10,0 mM/L) and (1,0mM/L), CMC-41 (1,0 mM/L), of MAD levels for CMG-41(10,0 mM/L) and CMJ-33 (10,0mM/L); for CAT by CMC-34 (10,0 mM/L) and CMG-41 (10,0 mM/L). Other CCC did not exhibit statistical difference on MAD, SOD and CAT levels.

**Conclusions:** The influence of studied CCC on the OS indices was selective and differentiate. Further studies are needed to evaluate the mechanisms of action in other biosystems and experiments.

**Keywords:** copper coordinative compounds, oxidative stress, *ex vivo* experiments.

**References:**

1. Gulea A., Roy J., Stavila V.et al. In vitro antileukemia, antibacterial and antifungal activities of some3d metal complexes: Chemical synthesis and structure activity relationships. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2008; 23(6):806-818.
2. Gulea A., Poirier D., Pahonţu E., Ţapcov V., Bejenari N., Roy J. *Inhibitori ai leucemiei mieloide umane în baza compuşilor coordinativi ai cuprului (II) cu saliciliden-tiosemicarbazide.* Brevet de invenţie MD3890, 2009. BOPI, 2009:4;35.
3. Pahontu Elena, Usataia Irina, Graur Vasilii et al. Synthesis, characterization, crystal structure of novel Cu (II), Co (III), Fe (III) and Cr (III) complexes with 2-hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone: Antimicrobial, antioxidant and in vitro antiproliferative activity // Appl Organometal Chem. 2019; e 4544.

*This study was supported by the State Program (2020-2023) of the Republic of Moldova (research grant No. 20.80009.5007.10).*

## ARTEMISININ PRODUCTION USING GENETIC AND METABOLOMIC ENGINEERING

Rakhmanov B., Imamkhodjaeva A., Ubaydullaeva Kh., Usmanov D.,
Mirzakhmedov M., Shermatov Sh., Buriev Z.
*Center of Genomics and Bioinformatics, Academy of Sciences of the Republic of Uzbekistan, Uzbekistan*
e-mail: bakhtiyor.rakhmanov@gmail.com

CZU:582.998.1:581.13:575.16 https://doi.org/10.52757/imb22.66

*Artemisia annua* L. also known as sweet wormwood is one of the most popular herbaceous medicinal plant with its therapeutic benefits and application in medicine, from which the substance artemisinin as a valuable for medicine is obtained, but artemisinin is found in a very small amount in A. annua. Its low amount in Artemisia annua makes artemisinin valuable and unique in the world pharmaceutical industry. Many factors influence the amount of artemisinin in a plant, including growing conditions, environment, climate, temperature, soil salinity, water supply, light, and harvest season.

*Artemisia annua* has become a profitable crop in the countries where artemisinin is considered to be the most sought-after substance and is grown on large areas along with other agricultural crops. Specially optimized agrotechnical measures for the cultivation of this plant have been developed, published as a manual, and widely implemented in practice. Lines and other varieties of the plant A. annua, which produces the most amount of artemisinin, were developed and research was carried out using marker-assisted breeding technology.

Currently, artemisinin extract from Artemisia remains the main source for medicine, and its concentration in this plant is 0.01–1.2% in dry weight, but its content is relatively higher in cultivated varieties. Artemisinin is the most effective agent used in the fight against malaria in the world, as well as its promising properties are reported to combat against several types of cancer, viral diseases like hepatitis, influenza, human immunodeficiency virus (HIV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes COVID-19 (coronavirus disease 2019).

Today, with the increase in the number of people affected by the diseases, the demand for this substance is also increasing sharply. Because of the low amount in A. annua plant researchers are trying to obtain artemisinin in more amounts in other organisms and plant species using genetic and metabolomic engineering. Several plants and agricultural crops, as well as by analyzing biosynthetic pathways of artemisinin and their responsible genes in our study we have successfully started initial steps of our project. Specific genetic vectors were developed and they were transformed into plant explants, and now we are studying their development in somatic embryogenesis in in vitro conditions in order to obtain whole transgenic plants carrying new vectors/genes.

# INFLUENCE OF SIDEROPHILIC NANOPARTICLES (Au, Fe, Co) ON ACTIVITY OF GLUCOSE OXIDASES OF PENICILLIUM GENUS

Semashko T.[1], Poladyan A.[2]
[1]*Institute of Microbiology of the National Academy of Sciences of Belarus, Belarus*
[2] *Yerevan State University, Armenia*
e-mail: tsemashko@mbio.bas-net.by

CZU:579.6 https://doi.org/10.52757/imb22.67

Nanotechnology is a high–tech industry aimed at studying atoms and molecules. Development in this field leads to revolutionary successes in medicine and electronics. Of particular interest is the production of metal nanoparticles and their application in biosensor technologies. However, the high cost of the most commonly used nanoparticles (Au- nanoparticles and Ag- nanoparticles) forces researchers to pay attention to other nanostructures based on metals (such as Fe, Pt, Au, Ag, Ni), metal alloys containing Pt, Au, Pb, Ir, Ru, Cu, Pd, and metal oxides (such as ZnO, CuO, $Cu_2O$, $MnO_2$, $TiO_2$, $CeO_2$, $SiO_2$, $ZrO_2$, $Fe_3O_4$). Biosensors constructed on the basis of the above-mentioned nanoparticles are characterized by high selectivity, sensitivity, fast response time, and stability.

The purpose of the work is to obtain siderophilic nanoparticles (Au, Fe, Co) and analyze their effect on the activity of glucose oxidases of *Penicillium* fungi.

Nanoparticles of metals Au, Fe and Co were obtained by chemical synthesis methods. The size of the obtained nanoparticles varied from 6 to 150 nm. The stability of nanoparticles has been verified. It was found that gold nanoparticles remained stable and did not require additional functionalization. In other cases, when Co and Fe nanoparticles were obtained, their tendency to form associations (aggregates and agglomerates) was manifested. Polyphenols contained in tea extract and permeate of *Penicillium adametzii* culture fluid were used to stabilize iron nanoparticles. It is shown that sodium stearate should be used to prevent agglomerates of cobalt nanoparticles. At the same time, the size of nanoparticles depends on the molar ratio of the studied compound and Co nanoparticles and varies from 50 to 150 nm.

Subsequently, the effect of the above nanoparticles on the activity of glucose oxidases of *Penicillium adametzii* and *P. funiculosum* was studied. It was shown that cobalt and iron nanoparticles had an inhibitory effect on enzyme activity in all concentrations studied. With an increase in the concentration used, the inactivation constant increased by 8-10 times. Activators of glucose oxidase activity were not detected in the experiments.

As for Au nanoparticles, it has been established that the toxic effect of nanoparticles is due not only to their size but also to the method of their production. Thus, when Au nanoparticles obtained by the Brast-Shifrin method were added to the enzymes (in a ratio of 1:100 solution), their activity decreased by 56-63 %. This is probably due to the presence of enzyme inhibitors (toluene, tetraoctylammonium bromide) in the colloidal gold solution. The best method for obtaining nanoparticles was the French method, in which sodium citrate was used as a reducing agent and stabilizer. When the Au nanoparticles obtained in this way with a size of 6-13 nm were added to the enzyme solution in a ratio of 1:100, and an increase in their activity was observed by 1.2-1.5 times. It should be noted that a decrease in the affinity of enzymes to the substrate was observed, however, the efficiency of glucose oxidation was not observed.

*The research was conducted within the framework of the project B21ARM-021 financially supported by the Belarusian Republican Foundation of Basic Investigations.*

# WHITE, GOLD and GREY Biotechnology

Gene-based Bioindustries,
Bioinformatics,
Nanobiotechnologies,
Classical fermentation,
and Bioprocess technology

**INFLUENCE OF THE BIO PRODUCT BioR ON THE LIPID COMPOSITION OF *STREPTOMYCES FRADIAE* CNMN-Ac-11 BIOMASS**

Bereziuc I., Burtseva S., Birsa M.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: ulia2032@mail.ru

CZU:582.232:577.182:63 https://doi.org/10.52757/imb22.68

Actinobacteria, including the genus *Streptomyces*, the most widespread in nature, are known for the wide range of bioactive substances they produce. Culture medium composition is crucial - the presence of certain components affects the composition of the obtained biomass, and directs the synthesis towards obtaining the necessary components.

Among the culture media offered for the cultivation of actinobacteria, mineral media are widely used - Dulaney, Pruss, Czapek with glucose or sucrose, etc., as well as complex or organic media in which the main source of carbon is flour (soybean, corn, etc.), as well as various additives (corn extract, molasses, baker's yeast, yeast hydrolyzate) and mineral salts. To obtain more biomass, reduce its cost, various components are added to obtain the desired effect. Actinobacteria are known for their ability to form lipids. Lipids perform many functions: they exhibit antibacterial, antioxidant, immunostimulating and antitumor properties, phospholipids regulate the activity of membrane enzymes.

As object of the study served strain *Streptomyces fradiae* CNMN-Ac-11, isolated from the soil of the central part of Republic of Moldova. To study the effect of nutrient medium compounds on the lipids and the fractional lipid producing, the BioR preparation, an extract of amino acids and peptides from *Arthrospira platensis* CNM-CB-02, was added to the culture medium at different concentrations. After cultivation, the biomass was separated from the culture liquid in a centrifuge (5000 rpm for 20 min). The amount of biomass was determined by the weight method. Next, intracellular lipids were extracted from the biomass by the Folch method with modifications. The qualitative and quantitative composition of lipids was determined by thin-layer chromatography on Sorbfil plates and densitometrically.

When cultivating the strain *Streptomyces fradiae* CNMN-Ac-11 on a modified nutrient medium, an increase in biomass productivity was noted. The maximum increase in the amount of biomass was observed when BioR was added at a concentration of 0.1%: by 18.2% more compared to the control sample. The study of the lipid composition of the biomass showed a lower content of lipids than in the control. Thus, the largest amount of lipids is contained in the biomass grown with the addition of the BioR preparation at a concentration of 0.1% to the R medium: the amount of lipids was 82.7% of the control sample.

The maximum amount of phospholipids was observed in lipid samples of the biomass of the strain grown on medium R with the addition of the substance at a concentration of 0.05% - 20.3% to total lipids. The largest amount of sterols was found in samples of total lipids of the biomass obtained by adding the substance to the medium at a concentration of 0.1%, and amounted to 14.7%. The amount of triglycerides was the highest in the lipid samples of the biomass of the strain growing on the R medium with the addition of the substance at a concentration of 1.0%, and amounted to 22.4% of total lipids.

Thus, the conducted studies have shown that in order to increase the productivity of the biomass of the strain *Streptomyces fradiae* CNMN-Ac-11 and the content of physiologically active lipid fractions (phospholipids and sterols) in it, the most optimal is the cultivation of the strain on the R complex medium with the addition of the BioR preparation at a concentration of 0.1-1.0%.

*The research was funded out within the project 20.80009.7007.09 (NARD).*

# SPIRULINA BIOMASS CONTAINING SILVER NANOPARTICLES – RAW AND SAFE MATERIAL FOR THE DEVELOPMENT OF MULTIPURPOSE REMEDIES

Chiriac T.,[1] Rudi L.,[1] Cepoi L.,[1] Chiriac V.,[2] Rotari I.,[1] Valuta A.,[1] Djur S.[1], Tasca I. [1]
[1] *Institute of Microbiology and Biotechnology, Chisinau, Republic of Moldova*
[2] *PI TL „UNIVERSUL"*
e-mail: chiriac.tv@gmail.com

CZU:582.232:615.322:579.61 https://doi.org/10.52757/imb22.69

Today, bionanotechnology represents one of the fastest growing areas *of* interdisciplinary research with applications in various fields. Along with the results obtained in the development of various models for the biosynthesis of nanoparticles, the results of using metal nanoparticles as stimulators of cell growth and biosynthetic processes of various biotechnological objects of industrial interest were noted.

Cyanobacterium *Arthrospira platensis* (spirulina) is well known as one of the most industrially used sources of various biomolecules with distinct biological properties. A number of nanoparticles, especially silver nanoparticles, have been shown to have a positive effect on cell growth and metabolism during spirulina cultivation. It has been established that the type, size, coating, and concentration of nanoparticles determine the nature of their effect on metabolic processes in this culture. At the same time, when using nanoparticles as biosynthesis stimulators, there is a possibility that they can affect the biological value of spirulina biomass as a raw material and, ultimately, the quality of end products. The conditions and parameters of spirulina cultivation process in the presence of silver nanoparticles in a polyethylene glycol coating have been determined, which ensure the production of safe and harmless additives from spirulina biomass without diminishing its biological value. In this case, spirulina cultivation was carried out on a nutrient medium containing (g/L): $NaNO_3$ - 2.5, $NaHCO_3$ - 8.0, NaCI - 1.0, $K_2SO_4$ -1.0, $Na_2HPO_4$ - 0.2, $MgSO_4 \bullet 7H_2O$ - 0.2, $H_3BO_3$ - 0.00286, $MnCI_2 \bullet 4H_2O$ - 0.00181, $CuSO_4 \bullet {}_5H_2O$ - 0.00008; $MoO_3$ - 0.000015, FeEDTA - 1.0 ml/L, at a temperature of 30- 32°C, pH 8.0-10.0 and illumination of 37-55 µM photons/$m^2$ under continuous lighting regime, for 6 days. On the 5th day of growth, polyethylene glycol-coated silver nanoparticles up to 5nm in size were added to spirulina culture at a concentration of 0.10 - 0.12 µM/L. At the end of the cultivation cycle, about 1.2 g/L of biomass (up to 30% more) and about 6% lipids (up to 28% more) were obtained. The content of malondialdehyde in biomass, as one of the main markers of toxicity in cells, did not exceed the level of this indicator in the biomass obtained by growing spirulina in the absence of nanoparticles (see Figure).

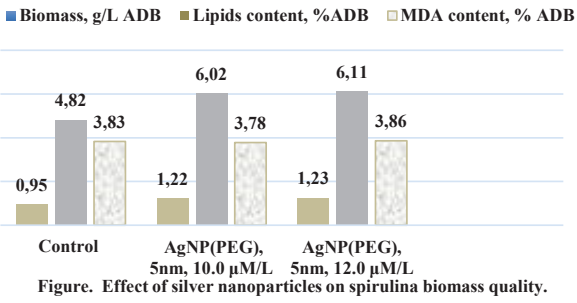


Figure. Effect of silver nanoparticles on spirulina biomass quality.

Thus, the addition of polyethylene glycol-coated silver nanoparticles at the end of the exponential growth phase reduced the contact time of spirulina cells with these nanoparticles. The age of the culture and the reduction of the contact time with nanoparticles were the determining factors that suppress the formation of free radicals and the excessive accumulation of end products of lipid peroxidation, which made it possible to obtain high-quality and safe biomass – raw material to develop products with multiple qualities.

*The scientific results were obtained within the project 20.80009.5007.05 „Biofunctionalized metal nanoparticles - obtaining using cyanobacteria and microalgae" funded by NARD, Republic of Moldova.*

# THE INFLUENCE OF SOME METAL NANOOXIDES ON THE EXOCELLULAR AMYLASE ACTIVITY OF *ASPERGILLUS NIGER* CNMN FD 06 MYCELIAL FUNGAL STRAIN

Condruc V., Ciloci A., Clapco S., Dvornina E., Labliuc S.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: alexandra.ciloci@gmail.com

CZU:582.282.123:574.2 https://doi.org/10.52757/imb22.70

In order to identify new innovative solutions for directing and increasing amylase biosynthesis in fungal strain *Aspergillus niger* CNMN FD 06, the effect of nanocomposites of Ti, Fe, Zn and Cu metals with different characteristics was evaluated.

In the research were included the nanoparticles (NPs) as follow: nanodioxide of Ti ($TiO_2$) with dimensions of 21 nm and <100 nm, titanium silicon oxide nanoparticles TiSiO4<50 nm, nanooxides $Fe_3O_4$ 50-100 nm, ZnO ≤50 nm and CuO <50 nm, as well as Cu metal (99,5%) with sizes of < 60-80 nm. The amylolytic activity of the strain *Aspergillus niger* CNMN FD 06 was monitored during the 5-7th days of cultivation – a period that corresponds to the maximum manifestation of amylase activity in the producer under submerged cultivation in classic conditions (control, without NPs). The nanocomposites were included in the culture medium in concentrations of 5 mg/L, 10 mg/L and 15 mg/L.

According to the obtained results, in all the experiments, the maximum of amylolytic activity in the control samples was established at the 6th day of cultivation and varied within 69.4 and 79.58 U/mL. In the experimental samples cultivated in the presence of Ti nanocomposites a stimulatory effect compared to the control of the same day was observed in the 5th day of cultivation. The amylolytic activity was superior to the control in all tested concentrations. In dependence of NPs concentration, the enzymatic activity exceeded the level of control by 17.64-24.06**%** in the case of $TiO_2$ nanodioxide of 21 nm, by 22.50-30.44 % in the variants with NPs $TiO_2$<100 nm and by 23.27-30.29 **%** – in the samples with titanium silicon oxide $TiSiO_4$<50 nm. At the same time, in the 5th day of cultivation a slight exceedance (by 1.72-2.34%) of the maximum control value (registered at 6th day of cultivation) was observed.

In the researches focused on the evaluation of copper nanoparticles effect, the maximum values of exocellular amylase activity in control samples were also found in the 6th day of cultivation, the activity being by 50.6% and 15.8% higher than in the 5th and, respectively, 7th day of cultivation. The activity of variants cultivated in the presence of NPs during 5th days was significantly higher compared to the control. The maximum stimulatory effect was found when minimal concentrations of the NPs were used. Thus, copper nanooxide with dimensions <50 nm ensured the increase of the enzymatic activity by 65.6% and 82.4%, respectively, at the concentration of 5 and 10 mg/L. When the NPs concentration was increased to 15 mg/L, the stimulatory effect is preserved, but it decreased to 55.8%. Copper nanoparticles (99.5%) with sizes of <60-80 nm ensure the increase of amylolytic activity by 60.7% (5 mg/L) and 65.6% (10 mg/L). Similarly, to the control variant, the maximum values of exocellular amylase activity in experimental samples were revealed on the 6th day of cultivation. The increase determined by nanoparticles was maintained only at the concentration of 10 mg/L for both NPs (CuO<50 nm and Cu (99.5%) <60-80 nm), constituting 9.3% and 10.5%, respectively.

According to the data, the $TiO_2$ nanodioxide with dimensions <100 nm, titanium silicon oxide $TiSiO_4$ nanoparticles with dimensions <50 nm, CuO nanooxide <50 nm and metallic nano-Cu (99.5%) with dimensions of <60-80 nm a recommend as stimulators of exocellular amylases biosynthesis in the micromycete *Aspergillus niger* CNMN FD 06 and can be used as a strategy of increasing the technological performance of the producer.

*The research was carried out within the State Program Project 2020-2023 of the Republic of Moldova with the number 20.80009.5007.28 "Development of new multifunctional materials and effective technologies for agriculture, medicine, technique and the educational system based on "s" and "d" metal complexes with polydentate ligands", funded by NARD.*

# ANALYSIS OF THE INTERGENIC REGION FOR THE PRESENCE OF REGULATORY MOTIFS IN *PSEUDOMONAS CHLORORAPHIS* SUBSP. *AURANTIACA*

Gerasimova T., Liaudanskaya A., Verameyenka K.
*Biology Department, Belarussian State University, Belarus*
e-mail: lastartes@mail.ru

CZU:606:579 https://doi.org/10.52757/imb22.71

As a result of a wild-type bacteria *Pseudomonas chlororaphis* subsp. *aurantiaca* B-162 (accession number CP050510.1) mutagenesis *P. chlororaphis* subsp. *aurantiaca* B-162/17 with an increased level of phenazine production was obtained. The quantity of phenazines in this mutant strain was almost three times higher than in the wild-type bacteria. Moreover, the B-162/17 strain was capable of producing phenazines on a minimal medium in contrast to the *P. chlororaphis* subsp. *aurantiaca* B-162. Phenazines are a group of heterocyclic nitrogen-containing compounds exhibiting a wide range of antibiotic properties, in particular, they have phytopathogenic and antitumor activity. Also, phenazines are widely used as pH indicators, components of microbial fuel cells and biofuel components in the biotechnology industry. Therefore, identification of mutations leading to phenazine overproduction on minimal nutrient medium becomes relevant.

Comparative analysis of wild-type and mutant genome sequences revealed potentially significant mutations in intergenic regions that can affect the level of phenazine synthesis. Using the SigmoID tool, the presence of motifs typical for the TetR and LuxR families of transcriptional regulator proteins was predicted in the intergenic region (coordinates 3443576 to 3443752 bp) located between genes expressing ABC transporter permease and LLM class flavin-dependent oxidoreductase.

To confirm the role of deletion which is located in described above intergenic region we decided to delete this region and analyze the resulting mutants. For this purpose, we have designed primers: CGGATCCTCCTTCCTCATCAAGCC (forward) and CGGATCCCAAAGGGCGTTGGTC (reverse), with BamHI restriction sites (highlighted in bold). The obtained PCR product was cloned into the pK18mob integrative suicide vector, which contains ampicillin and kanamycin resistance genes. The polylinker is located inside the ampicillin resistance gene. After entering the bacterial cell this genetic construction recombines with the chromosome and disrupts the integrity of the target sequence due to the presence of the homology region in it.

Using $CaCl_2$ transformation, the resulting vector was inserted into the *Escherichia coli* BW19851 bacteria, which are capable of conjugation. For this purpose, the donor bacteria *E. coli* BW19851 with the vector and the recipient *P. chlororaphis* subs. *aurantiaca* B-162 were mixed in a 70:30 ratio. The mixture of donor and recipient cells was incubated in a liquid LB medium for one hour and then, after precipitation, was transferred to the agar medium in the shape of medallions and incubated for 24 h at 28 °C. Cultured cells were transferred in a selective agar medium containing 50 μ/ml kanamycin and 50 μ/ml ampicillin (used to remove donor cells) and then incubated for 42 h at 28°C. The grown colonies were analyzed.

Analysis of *P. chlororaphis* subs. *aurantiaca* B-162 with deleted intergenic region showed that the phenazine level was higher than in wild-type *P. chlororaphis* subs. *aurantiaca* B-162 but lower than in *P. chlororaphis* subs. *aurantiaca* B-162/17. In this regard, the analyzed intergenic region contains regulatory motifs capable of affecting phenazine biosynthesis and excretion from the cell.

# INFLUENCE OF GOLD AND SILVER NANOPARTICLES ON THE SYNTHESIS OF PHYCOBILIPROTEINS IN RED MICROALGA *PORPHYRIDIUM CRUENTUM*

Rudi L., Chiriac T., Cepoi L., Valuta A., Djur S., Miscu V., Codreanu S., Tasca V., Rotari M.
*Institute of Microbiology and Biotechnology, Republic of Moldova*
e-mail: rudiludmila@gmail.com

CZU:57.044:582.26/.27:546.57 https://doi.org/10.52757/imb22.72

The biological properties of nanoparticles are determined by their size, shape, and surface characteristics. The study of the effect of nanoparticles on microalgae as biotechnological objects is one of the topical areas of modern research. Similar to the action of complex chemical compounds, the stimulating nature of the action of nanoparticles is based on reversible oxidative stress caused by the mechanisms of adaptation of a microalgae culture to cultivation conditions. Phycobiliprotein pigments, as the major light-harvesting pigments of red microalga *Porphyridium cruentum*, are involved in the cellular response to the presence of nanoparticles in the cultivation medium.

The influence of 10 and 20 nm citrate-stabilized gold and silver nanoparticles on the synthesis of phycobiliproteins in red alga *P. cruentum* was determined. Nanoparticles were added to the culture medium from the first day of the life cycle. At the end of the experiments, an increase in the content of phycobiliproteins in the biomass was determined due to the cellular response of microalgae, which manifested itself depending on the size of nanoparticles and their concentration.

AgNP(Citrate) 10 nm in size showed a strong direct correlation (r=0.855) between the concentration of nanoparticles in the cultivation medium and the content of phycobiliproteins in microalgal biomass. Thus, concentrations of 0.025, 0.05 and 0.25 μM stimulated the accumulation of phycobiliproteins in *P. cruentum* biomass by 18-29%. In the case of a concentration of 0.5 μM AgNPs, an increase in phycobiliproteins in algal biomass by almost 64% was estimated compared to the control, reaching a level of the content of these biologically active compounds in porphyridium biomass of 21%.

A similar effect was recorded for AuNP(Citrate) 10 nm in size. Concentrations of 0.05-0.1 μM of these nanoparticles increased the content of phycobiliproteins in porphyridium biomass by 26 - 28%. In this case, a strong direct correlation was also established between the concentration of nanoparticles and the values of phycobiliproteins, Pearson's correlation coefficient was r=0.812.

A response to stimulate phycobiliprotein synthesis was also established for AuNP(Citrate) and AgNP(Citrate) with a size of 20 nm, in this case showing dose-dependent reverse effects. In the case of application of 20 nm AgNP(Citrate) nanoparticles, stimulating concentrations ranged from 0.001 to 0.25 μM with an increase in the content of phycobiliproteins in biomass by 26-49%. The concentration of 0.5 μM AgNPs slightly enhanced phycobiliproteins in algal biomass. The correlation between the concentration of 20 nm AgNP(Citrate) nanoparticles in the culture medium and the content of phycobiliproteins in porphyridium biomass in this case revealed a moderately negative predictive relationship (r=-0.573).

For AuNP(Citrate) of 20 nm in diameter, a strong negative correlation (r=-0.719) was found between the concentration of nanoparticles in the culture medium and the content of phycobiliproteins in porphyridium biomass. It should be noted that at concentrations of AuNP(Citrate) of 20 nm in size in the range of 0.0025-0.02 nM, the increase in the content of phycobiliproteins in porphyridium biomass was 42 - 48% compared to control sample.

Thus, the stimulating effect of citrate-stabilized gold and silver nanoparticles in the tested sizes and within the selected concentrations was the result of their involvement in the biosynthetic activity of algae cells and depended on the nanoparticle size and concentration. The type of nanoparticles is a little determining factor for the synthesis and, accordingly, the accumulation of phycobiliproteins, which changed as a result of the response of microalgae cells to stress induced by the composition of the cultivation medium.

*The scientific results were obtained within the project 20.80009.5007.05 „Biofunctionalized metal nanoparticles - obtaining using cyanobacteria and microalgae" funded by NARD, Republic of Moldova.*

# COMPARISON OF PHENYLALANINE PRODUCTION LEVELS IN *P. CHLORORAPHIS* SUBSP. *AURANTIACA* PHENAZINE PRODUCING STRAINS

Kachan V., Liaudanskaya A., Verameyenka K.
*Biology Department, Belarussian State University, Belarus*
e-mail: bio.kachanVI@bsu.by

CZU:579.841.11:577.2 https://doi.org/10.52757/imb22.73

Phenazines designate a group of nitrogen-containing heterocyclic pigmented secondary metabolites with the pronounced capacity to inhibit plant pathogens. Phenazines are secreted by a diversity of bacteria, particularly *Pseudomonas* strains. Phenazines have received researchers' interest due to their wide-ranging antibiotic properties and have been widely used in the biological control of various fungal phytopathogens. Besides phenazines are considered potential anti-cancer agents.

*Pseudomonas chlororaphis* subsp. *aurantiaca* bacteria are producers of various biologically active substances, including phenazines. *P. chlororaphis* subsp. *aurantiaca* B-162 were obtained from the collection of microorganisms from the Genetics Department of the Belarusian State University. This strain demonstrated stable phenazine production. This wild-type strain was further used for the mutagenesis and selection of highly productive producer strains. Two mutant producer strains B-162/255 and B-162/17 was derived following sequential mutagenesis coupled with the selection on resistance to the toxic analog of aromatic amino acid.

Genomic sequencing of these strains revealed several mutations that could potentially cause an increase in phenazine production in *Pseudomonas* bacteria. In particular, a point mutation was found in strain B-162/255 in the gene encoding phenylalanine hydroxylase transcriptional activator PhhR involved in the degradation and homeostasis of phenylalanine, which in turn is a negative regulator of DAHP synthase implicated in the catalysis of biochemical reactions leading to the phenazines synthesis.

The purpose of the research was to evaluate the effect of a mutation in the *phhR* gene on phenylalanine production by mutant strains.

This experiment was conducted with *P. chlororaphis* subsp. *aurantiaca* strains B-162, B-162/255, and B-162/17 were cultivated on a minimal medium (M9) and a production media. After 12 h, 18 h, 1 day, 2 days, and 3 days of incubation, which correspond to the exponential stage of culture growth, the level of phenylalanine production was determined by a modified spectrophotofluorometric method of McCaman and Robins.

The wild type strain B-162 is characterized by minimal phenylalanine production level on a production medium among all three strains. By 12 hours of cultivation on a minimal medium, strain B-162 accumulates 3.8 times less phenylalanine than B-162/255 and 6 times less than B-162/17. The mutant strain B-162/255 is characterized by an increase in phenylalanine production by the third day of cultivation. The accumulation of phenylalanine by strain B-162/17 during cultivation on a production medium gradually decreases during the first day and increases again by the third.

In general, phenylalanine production for all three strains is higher on a minimal medium (M9). Based on the obtained results, it was concluded that the maximum productivity values for phenylalanine are characteristic of strain B-162/255. This correlates with the hypotheses that the mutation in the *phhR* gene affects the production of phenylalanine by *P. chlororaphis* subsp. *aurantiaca* B-162/255.

# SELENIUM-ENRICHED FODDER YEAST: PRODUCTION AND APPLICATION IN STOCK BREEDING

Moroz I.[1], Sapunova L.[1], Pauliuk A.[1], Shareika M.[2]
[1]*Institute of Microbiology, NAS Belarus, Belarus*
[2]*Vitebsk State Academy of Veterinary Medicine, Belarus*
e-mail: irmorz@gmail.com

CZU:636.087.7 https://doi.org/10.52757/imb22.74

The growing market demand for selenium-containing food and feed products is accounted for by their vital role in ensuring normal life activities of humans and animals. This microelement displays antioxidant, immunomodulating and detoxifying properties; it is directly involved in formation of active sites of some enzymes. The shortage of selenium results in disbalance of metabolic processes, growth retardation, degenerative changes in organs and tissues, reproductive dysfunction.

So far to upgrade fodder selenium was added as inorganic compounds distinguished by poor assimilation rate, inadequate biological efficiency and high toxicity. Lately organic Se-containing substances of synthetic and microbial origin came into use. The latter are represented by selenium-accumulating yeast species where this trace element is localized in readily digestible, bioaccessible form. Moreover, selenium-enriched yeast contains high-quality protein, minerals, vitamins and essential amino acids. Application of selenium-supplemented fodder yeast raises metabolic, biochemical and immune status of reared animals, improves digestion, diminishes risks of pathologies caused by malfunction of gastrointestinal system and metabolic disorders due to selenium deficiency, increases productivity, saves feed expense, enhances survival rate of farm stock and poultry.

To manufacture fodder premixes upgraded with organic selenium the adapted to the microelement yeasts of genera *Saccharomyces* and *Candida* find most wide-spread use. They lay the basis for readily available, economically grounded and ecologically safe technology of commercial production of organic selenium additives.

The top manufactures of selenium-upgraded alimentary and fodder yeasts in the world are the following companies: Pharma Nord, Garuda, Angel Yeast, Lesaffre, Alltech, Miro Chembiotech, Lallemand, ADM, ABF and some others. According to the Global Market Insights Inc. report, the overall volume of this biotechnological commodity by 2027 is expected to surpass 285 mln US dollars. The forecast for global demard of Se-enriched fodder yeast is likely to exceed 175 mln US dollars. The rising consumption of this market product was provoked mainly by imposed ban in European Union on introduction of antibiotics and growth promoters into feed rations.

There is no production of similar products in Belarus, while in the conditions of intensive development of animal husbandry and moderate deficiency of trace elements in water and soil, the demand for selenium-containing feed additives in the country is increasing, as well as in the world as a whole. Now a pilot-plant process for production of Se-enriched fodder yeast based on adapted to the microelement strain *Candida* sp. 4-ASe is being developed at the Institute of Microbiology, National Academy of Sciences of Belarus.

The preliminary trials of pilot batch of feed additive into the rations of sucking calves indicate 4,7–7,3% increase of average daily live body weight gains as compared with the control group. It was also found that Se-yeast consumption by sucking calves results in recovery of gut microbiota represented by bacteria of genera *Lactobacillus, Bifidobacterium, Bacillus, Clostridium, Enterococcus*, whereas the ratio of coliform bacteria (*Escherichia coli*) tends to decline.

Generally, supply of new additive into feed rations of sucking calves is conducive to their physical and reproductive health, normalizes composition of intestinal microbiota, raises the yield and quality of farm produce, contributes to output of foodstuffs intended for prevention of diseases associated with selenium deficiency.

# CHARACTERIZATION OF RECOMBINANT AMINOACYLASE FROM *ESCHERICHIA COLI*

Yepremyan H.
*Scientific and Production Center "Armbiotechnology" of NAS RA, Yerevan, Armenia*
e-mail: hasmikyepremyan31@gmail.com

CZU:579.6:[577.1+579.842] https://doi.org/10.52757/imb22.75

## Introduction

Aminoacylases – (N-acylamino acid amidohydrolase, EC 3.5.1,14) catalyzes N-acylated amino asids to yield the acyl group and the corresponding amino acid. Acylases are widespread in nature. This enzyme is found in the kidneys of animals and is produced by some fungi, yeast and other microorganisms. Most isolated aminoacylases show a substrate specificity towards N-acetyl-L-amino acids, including ones isolated from hog kidney [1], *Aspergillus oryzae* [2], *Alcaligenes denitrificans* DA181 [3], *Bacillus stearotermophilus* [4], *Lactococcus lactis* MG1363 [5], and *Burkholderia* sp. strain LP5_18B [ 6]. In addition, the genes for enzymes such as those from *Bacillus stearotermophilus, Lactococcus lactis* MG1363 and *Burkholderia* sp. strain LP5_18B have recently been analyzed. In practice, aminoacylases are widely used on an industrial scale to obtain optically active amino acids [7].

**The aim** of this work is to study the substrate specificity of the recombinant inracellular aminoacylase from *Escherichia coli* LGE 36 and some of its properties.

## Materials and methods

Recombinant strain-producer of aminoacylase *E. coli* LGE 36 [ 8] was used in this work. The cells were grown in M9 minimal medium with supplements at $37^0$C. The cells were disintegrated by sonication in 100 mM Na, K-phosphate buffer, pH7,0, containing 0,2mM $CoCI_2$ and 1 mM phenylmethylsulfonyl fluoride (PMSF). The cell debris was removed by centrifugation and the resulting supernatant was used for experiments.

The aminoacylase activity was determined by the modified method of Gade and Brown [9]. In the reaction medium of 200 µl of final volume, containing 100 mM Na, K-phosphate buffer, pH7,0, 0,2mM $CoCI_2$, 40 mM N-acetyl-D, L-methionine and enzyme in the required amount, at $37^0$C. The unit of acylase activity was defined as the amount of the enzyme catalyzing the formation of 1 µmol of L-amino acid per min. Protein was determined by method of Lowry [10].

All chemicals were purchased from Sigma-Aldrich and Reanal.

## Results and Discussion

Earlier we have developed the method for obtaining (isolation and purification) the recombinant intracellular aminoacylase of *E. coli* by twofold ion exchange chromatography on DEAE-Cellulose [11]. The investigated enzyme has shown that it is a dimer, composed of two identical subunits with the molecular mass of 42 kDa for each. It has a pH optimum of 7,0.

The substrate specificity of the aminoacylases from various soucers has been briefly described, but we have reexamined the specificity of the enzyme for a wide variety of N-acyl amino asids and derivatives. A number of N-acyled amino acids were tested as possible substrates for aminoacylase preparations. Table 1 summarizes the substrate specificity of the recombinant intracellular aminoacylase from *E. coli* LGE36. The enzyme from *E. coli* catalyzed the hydrolysis of most of the α-N-acetyl-L-amino acids. The recombinant aminoacylase showed high activities, particullarly towards N-acylated amino acids, such as N- acetyl- L-methionine, N- acetyl- L- alanine, N- acetyl- L-valine, N-acetyl-L-leucine, while activities for N-acetyl-L-serine was low. It had very low activity for N-acetyl -L-aspartic acid and N-acetyl-L-glutamic acid. Under the conditions employed only α-N-acetyl-L-ornithine and α-N-acetyl-L-lysine were found to be deacetylated at a higher rate. The relative activity of hydrolysis of α-N-acetyl-L-lysine is threefold efficient, whereas that of α-N-acetyl-L-ornithine is fivefold better than N-acetyl-L-methionine substrate. Perhaps, the best substrates for the aminoacylase are α-N-acetyl- L-ornithine and α-N- acetyl-L-lysine.

The enzyme exhibits absolute stereospecificity for acylated L-amino acids, being unable to hydrolyze N-acyl-D-amino acids. The rate of deacylation of N-acylated amino acid substrates is sensitive to the nature of the amino acid, with amino acids containing aromatic side chains, for example, N-acetyl-L-phenylalanine showing no activity. When incubated with this enzyme the chloroacetyl derivatives of

phenylalanine were moderately hydrolyzed. Besides acyl amino acids, hydrolytic activity (peptidase activity) of the recombinant aminoacylase from *E. coli* towards dipepdides such as glycyl-L-methionine was also studied. As a result, it was revealed that glycyl-L-methionine was rapidly hydrolyzed.

**Table 1**. **Substrate specificity of the intracellular recombinant aminoacylase from *E. coli* LGE 36.**

| Substrate | Relative activity*, % |
|---|---|
| N-Acetyl-D,L-methionine | 100 |
| N-Acetyl-D-methionine | 0 |
| N-Acetyl-D,L- alanine | 64 |
| N-Acetyl-L -alanine | 70 |
| N-Acetyl-D -alanine | 0 |
| N-Acetyl-D,L-valine | 60 |
| N -Acetyl-D-valine | 0 |
| N-Acetyl-D,L-leucine | 80 |
| N-Acetyl-D-leucine | 0 |
| N-Acetyl-D,L-serine | 35 |
| N-Acetyl-L-aspartic acid | 30 |
| N-Acetyl -L-glutamic acd | 15 |
| a-N-Acetyl-L-lysine | 300 |
| a-N-Acetyl-L-ornithine | 500 |
| N-Acetyl-L-phenylalanine | 0 |
| N-Chloroacetyl-L-phenylalanine | 28 |
| Glycyl-L-methionine | 200 |

*100% of relative activity corresponded to 1050 U/mg of the specific activity of the enzyme.

A number of studies have reported data on the substrate specificity of aminoacylases from various sources. Aminoacylases such as hog kidney [1] and bovine liver [12] show a very narrow range of substrate specificity. Aminoacylase from *Aspergillus oryzae* [2], which is produced industrially, shows substrate specificity for N-acetyl -L-phenylalanine, N-acetyl -L-methionine, N-acetyl -L-tryptophan, N-acetyl -L-alanine, but its specific activity is very low. Aminoacylases from *Alcaligenes denitrificans* DA181 [3] and *Pseudomonas maltophila* B1 [12] show high specific activity, but the substrate specificity is narrow. The enzyme from *A. denitrificans* DA181 preferentially hydrolyses N-acetyl -L-alanine and N-acetyl -L-valine. The enzymes from *B. stearotermophillus* and *L. lactis* MG1363 have substrate specificity towards N-acetyl -L-amino acids with hydrophobic amino acid residues. Obtained data indicated that the recombinant intracellular aminoacylases from *E. coli* show wider substrate specificity than those from the other sources, as described above. The investigated enzyme effectively catalyzes the hydrolysis of most N-acetyl -L-amino acids, including those of basic amino asid. Among the best substrates, we find acetyl derivatives of two basic amino acids – L-ornithine and L- lysine.

**Conclusions**

In this study, we report on characterization of the recombinant intracellular aminoacylase from *E. coli* LGE36 with high levels of aminoacylase activity and wide substrate specificity. The *E. coli* aminoacylase showed higher activity towards α-N-acetyl-L-ornithine and α-N-acetyl-L-lysine rather than towards N-acetyl-L-methionine. The comparison of the substrate specificity of the recombinant intracellular aminoacylase from *E. coli* with other members of the aminoacylase family suggests an origin of the obtained enzyme.

**References:**

1. Birnbaum S.M., Levintow L., Kingsley R.B. and Greenstein J.P. 1952, *J.Biol.Chem*.,194,455-470.
2. Gentzen I, Loffler H.G. and Schneider F. 1980 Z. Naturforsch., 35 c, 544-550.
3. Yang Y.B., Hu H.L., Chang M.C., Li H. and TsaiY.C., 1994*, Biosci. Biotechnol. Biochem*., 58, 204-205.
4. Sakanyan V., Desmarez L., Legrain C., Charlier D., Mett I., Kochikya A., Savchenko A., Boyen A., Falmagne P., Pierard A. and Glansdorff N.1993, *Appl. Environ. Micbiol*., 59, 3878-3888.
5. Curley P., Van Der Does c., Driessen A. J., and Van Sinderen D. 2003, *Arch.Microbiol*., 179, 402-408.
6. Takakura Ya. and Asano Ya. 2019, *Biosci. Biotechnol. Biochem*., v. 83, No. 10, 1964-1973.
7. Aggarwal S., Sahni S. 2012*, International Conference on Enviromental, Biochemical and Biotechnology IPCBEE*, 41, 18-22.
8. Mett I. L., Mett A.L., Kochikyan A.V., Sakanyan V. A. and Ivanov A.S. 1987, *USSR patent* 1, 510, 360.
9. Gade W., Brown J.L. 1981*, Biochem. Biophys. Acta*, 662, 86-93.
10. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. 1951, *J.Biol. Chem.,* 193, 265-275.
11. Yepremyan H. S., Artsruni G.K. 2011*, Abstracts of the 6$^{th}$ Moscow Intarnational Congress "Biotechnology: state of the art and properties of development",* Moscow, March 16-20,128.
12. Wakayama M., Shiiba E., Sakai K., and Moriguchi M. 1998, *J. Ferment. Bioeng*., 85, 278-282.

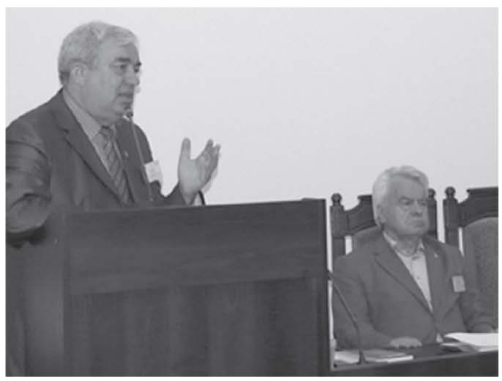
# Previous conferences

## 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:

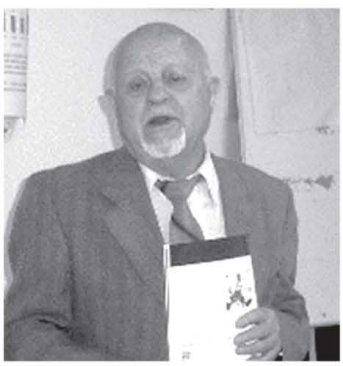
1. Microbiological techniques/biotechnologies for agriculture.
2. Microbiological techniques/ biotechnologies for medicine.
3. Microbiological techniques/ biotechnologies for environment.



# 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)







# International Scientific Conference on Microbial BIOTECHNOLOGY (4th EDITION)

The Conference was held in Chisinau, Republic of Moldova on 11-12 October 2018. The Conference was attended by 270 participants from Moldova, Romania, Czech Republic, Ukraine, India, Belarus, Russia, Kazakhstan, Pakistan 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

Djur S. https://orcid.org/0000-0001-8093-3510; *102, 105*
Dudnicenco T. https://orcid.org/0000-0003-0484-6372; *31*
Dvornina E. https://orcid.org/0000-0002-0015-6131; *103*
Efremova N. https://orcid.org/0000-0002-9664-346X; *6, 27, 29 82*
Elenciuc D. https://orcid.org/0000-0002-5090-5057; *86*
Enache M. https://orcid.org/0000-0003-2070-8439; *2, 6, 10, 25, 32*
Enciu V. https://orcid.org/0000-0002-1941-1323; *78, 85*
Farsiyan A. *90*
Gaina B. *77*
Garbuzneac A. *33*
Gerasimova T. *104*
Ghinda S. *22, 91*
Ghochikyan V. *53,54, 55*
Gomoiu I. *10*
Grozdov D. https://orcid.org/0000-0002-0297-324X; *9, 19, 65*
Grumezescu V. *21*
Gudumac V. https://orcid.org/0000-0001-9773-1878; *83*
Gutu N. *6, 89*
Gutul T. *11, 52*
Hlushen A. *39, 50*
Hubchyk K. *34, 50*
Iatsko I. https://orcid.org/0000-0003-0757-7884; *84*
Imamkhodjaeva A. *18, 98*
Indoitu D. https://orcid.org/0000-0003-4190-7816; *6, 35*
Israyelyan A. https://orcid.org/0000-0002-6789-9853; *6, 48, 90*
Ivantoc N. *38*
Jurminskaia O. *70, 73*
Kachan V. *106*
Karapetyan Zh. https://orcid.org/0000-0002-0226-0645; *53, 54, 55*
Keleshyan S. https://orcid.org/0000-0003-0586-4863; *53, 54, 55*
Khomidjonova S. *64*
Kolodrevski O. https://orcid.org/0000-0002-2102-5886; *88*
Labliuc S. https://orcid.org/0000-0002-5692-5649; *103*
Larion A. *88*
Lazutin N. *17*
Lesnic E. https://orcid.org/0000-0002-4259-0227; *5, 22, 91, 96, 97*
Liaudanskaya A. https://orcid.org/0000-0002-3735-537X; *104, 106*
Lucaci I. *10, 25, 32*
Lungu A. *6, 36, 51*
Lupascu L. https://orcid.org/0000-0001-5006-5265; *37*
Lupascu T. https://orcid.org/0000-0001-5913-7691; *37*
Macari V. https://orcid.org/0000-0002-8072-4150; *5, 83*
Malic A. https://orcid.org/0000-0002-5216-6470; *6, 96*
Mamaliga V. https://orcid.org/0000-0001-5658-6571; *42, 63*
Melkonyan L. *53, 54, 55*
Melnic G. *88*
Mereniuc L. https://orcid.org/0000-0002-5222-7317; *11*
Mirzakhmedov M. *18, 98*
Miscu V. https://orcid.org/0000-0003-0774-2085; *2, 84, 105*
Moldovan A. https://orcid.org/0000-0001-8829-6640; *5, 38*
Moldovan C. https://orcid.org/0000-0003-1634-0344; *6, 71*
Moroz I. *6, 44, 107*
Moroz M. *26*

Sîtnic F. https://orcid.org/0000-0002-4531-2791; *11*
Slanina V. https://orcid.org/0000-0002-9833-7933; *28*
Sprincean A. https://orcid.org/0000-0002-3725-2872; *27*
Stan M.S. *21*
Stici V. *6, 78, 85*
Stingaci A. https://orcid.org/0000-0001-6621-9919; *5, 51*
Sturza R. https://orcid.org/0000-0002-2412-5874; *77*
Sudakova K. *20*
Talambuta N. https://orcid.org/0000-0003-3740-4335; *88*
Tasca I. https://orcid.org/0000-0002-4053-3296; *102*
TascaV. https://orcid.org/0000-0002-3955-011X; *105*
Timbaliuc N. https://orcid.org/0000-0002-5240-4651; *37*
Timus I. https://orcid.org/0000-0001-5160-3467; *72*
Tkhruni F. *48, 90*
Todiras V. https://orcid.org/0000-0002-3554-0512; *41, 52*
Tofan E. https://orcid.org/0000-0002-0186-4391; *27, 29, 82*
Toplaghaltsyan A. https://orcid.org/0000-0002-6819-7351; *53, 54, 55*
Tozar T. https://orcid.org/0000-0002-5953-9241; *21*
Trofim A. https://orcid.org/0000-0003-4557-9602; *6, 74, 86*
Tsarukyan G. *53, 54, 55*
Turcan O. https://orcid.org/0000-0002-7103-5986; *61, 62, 72, 74*
Ubaydullaeva Kh. *18, 98*
Ungureanu (Negut) I. https://orcid.org/0000-0003-4038-7548; *5, 21*
Usmanov D. *18, 98*
Valuta A. https://orcid.org/0000-0003-1233-9362; *6, 102, 105*
Verameyenka K. *104, 106*
Vergel K. *19*
Vlad A. *25, 32*
Voinescu A. https://orcid.org/0000-0001-7529-2907; *42, 63*
Vorona V. https://orcid.org/0000-0002-0729-0464; *5, 40, 42, 63*
Yepremyan H. *108*
Yushin N. https://orcid.org/0000-0001-5650-4337; *5, 65*
Zakiryaeva S. https://orcid.org/0000-0002-3309-1258; *5, 64*
Zamornea M. https://orcid.org/0000-0001-8987-3390; *88*
Zavtoni P. https://orcid.org/0000-0003-1841-0086; *51*
Zaynitdinova L. https://orcid.org/0000-0001-9638-6347; *17*
Zhukouskaya L. *5, 17, 20, 66*
Zinicovscaia I. https://orcid.org/0000-0003-0820-887X; *5, 9, 19, 65*
Zosim L. https://orcid.org/0000-0003-0510-8064; *6, 74, 86*