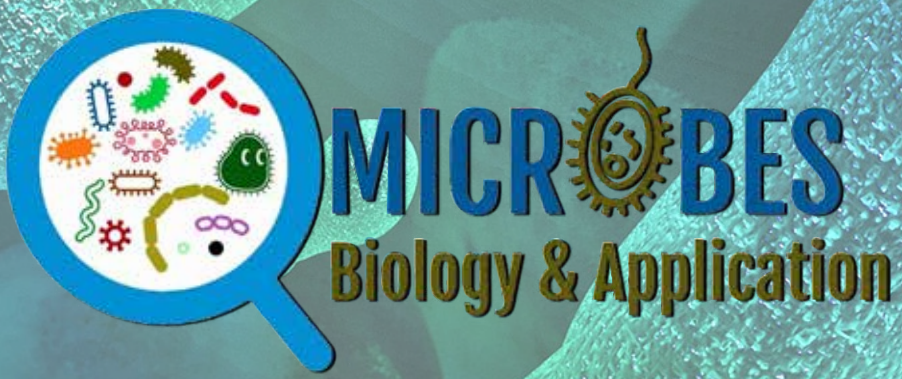
International Conference
"MICROBES: BIOLOGY&APPLICATION"



BOOK OF ABSTRACTS

October 9-11, 2019
Yerevan, Armenia

International Conference
“MICROBES: BIOLOGY&APPLICATION”

Международная конференция
«МИКРОБЫ: БИОЛОГИЯ И ПРИМЕНЕНИЕ»

Միջազգային գիտաժողով
«ՄԱՆՐԷՆԵՐ. ԿԵՆՍԱԲԱՆՈՒԹՅՈՒՆԸ ԵՎ ԿԻՐԱՌՈՒԹՅՈՒՆԸ»

BOOK OF ABSTRACTS
СБОРНИК ТЕЗИСОВ
ԹԵԶԻՍՆԵՐԻ ՀԱՎԱՔԱԾՈՒ

October 9-11, 2019, Yerevan, Armenia
9-11 Октября, 2019, Ереван, Армения
Հոկտեմբերի 9-11, 2019, Երևան, Հայաստան

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“MICROBES: BIOLOGY&APPLICATION”
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«МИКРОБЫ: БИОЛОГИЯ И ПРИМЕНЕНИЕ»
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Dear Colleagues,

It is our great pleasure to invite you to the International Scientific Conference "Microbes: Biology & Application" to be held in Yerevan, Armenia from 9-11 October 2019.
The Conference is organized by the Armenian Microbiological Association together with SPC "Armbiotechnology" NAS of Armenia by the support of FEMS and VIPECO AM LLC.

The aim of the Microbes: Biology & Application International Conference is to present the latest research results in the field of Microbiology in the light of practical needs and the prospective use of scientific achievements for promoting the cooperation between the academics, researchers and scientists. The Microbes: Biology & Application International Conference encompasses the following scientific disciplines: Microbial Ecology; Environmental Protection; Waste Management, Biosafety and Health, Biofertilizers, Food Safety, New Biotechnologies. This Conference is to exchange ideas and to strengthen cooperation between participants, as well as to identify the condition and direction of development of scientific relations in the field of Microbiology and the challenges and problems associated with their implementation.

The Conference will take place in Yerevan, Armenia. The Conference venue is Scientific and Production Center "Armbiotechnology" of the National Academy of Sciences of Armenia.

We will appreciate your interest in our Conference and look forward to seeing you in Armenia.

Sincerely,

Dr. Narine Vardanyan

AMA President, FEMS Delegate

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Untapped microbial composition of acidic soil of northeast India for potential agriculture application

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Microbes are one of the most vital components of our ecosystem. Without microbes survival is questionable to all the living beings on this earth. Therefore, the study of microbes in different strata is important and crucial. The composition of microbes in different environment varies and it is influenced by numbers of factors present in the soil. Among them, pH of the soil is one of the determining factors for changing the solubility of different metal ions, nutrient availability and various physical properties. It has been shown that the composition and diversity of soil bacterial communities are often strongly correlated with soil pH. Agricultural soil is such a hotspot of anthropogenic disturbance due to the long-term input of agricultural chemicals and pollutants, resulting in significant changes in soil characteristics such as acidification and attenuation of soil fertility. However, metagenomic study of the soil revealed the microbiome structure and it helps us to understand the microbiome and its functions more precisely. In this study, we are trying to understand the composition of microbial community in the acidic soil of some important vegetable growing fields of Northeast India and its potential application in the agricultural field.

Translocation of microorganisms with tumor processes

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The problems of carcinogenesis is one of the most important in modern medicine. Concomitant infections can often cause regression of the tumor, and to some extent this phenomenon is caused by the translocation of conditionally pathogenic microbes and the action of their toxins, cytokines and other factors. In particular, the American scientist Shire and his colleagues isolated the active substance lipopolysaccharide and showed that in response to it, a factor causing hemorrhagic necrosis of the tumor was formed in the serum of mice. Red blood cells were obtained in patients with acute leukemia. A suspension of *Escherichia coli* was added to a certain part of the erythrocyte sediment *in vitro*, and another mixture of physiological saline (control) was added to it. After a 2-hour incubation, suspensions were centrifuged, the resulting precipitates were fixed with 2.5% glutaraldehyde solution prepared in 0.1 M phosphate buffer with pH 7.2-7.4, fixed with 1% OsO_4 solution in the same buffer and processed using a common electron microscopy technique. Finished epon-araldite blocks were cut on the Austrian ultratome of the company Ultrasonic, contrasted sections were scanned using a BS 613 electron microscope from Tesla. In a macroorganism, the negative effects of translocation occur only when massive bacteremia develops, and in the process of bacterial migration specific clones of *E. coli* with a complex of properties of protective and aggressive orientation are involved: increased serous resistance, factors of bacterial persistence, toxins, etc. As a result of the studies, data on the functioning of the "bacterium-erythrocyte" system were obtained, taking into account the level of expression of the properties of microorganisms and their intra-erythrocyte effect. The phenomenon of intraerythrocyte interaction of bacteria was established.

Polysorbates biodegradation and the plasmid stability in soil opportunistic pathogenic strains of *Stenotrophomonas* and *Pseudomonas*

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Multidrug resistance is one of the well-known properties of *Pseudomonas* and *Stenotrophomonas*, isolated from clinics. But the native strains of these genera isolated from soil have the wide spectrum of resistance to antibiotics of II and III generation. This property can be transferred through plasmids to other Gram-negative bacteria too. This problem is not only of medical but also ecological significance. It is well known that Pseudomonas and Stenotrophomonas are highly adaptive microorganisms. They have a wide diversity of enzymes and biochemical pathways of biodegradation of various native substances and xenobiotics. This research is focused on study of biodegradation potential of more than 50 strains of 7 species and 3 subspecies of mentioned bacteria. Antibiotic resistance is a part of one system used by microorganisms to adapt to changing environment. It is important for understanding the mechanism of *Pseudomonas* and *Stenotrophomonas* resistance plasmids stability, and for solving modern ecological problems connected with pathogen appearance and xenobiotic biodegradation processes in soil. It was revealed that in polysorbate degradation 5 different lipases were involved. The enzymes can be localized both on plasmids and chromosomes. Probably, some lipases play a key role in stability of antibiotic resistance plasmid in some strains of *Pseudomonas maltophilia* and *Pseudomonas taetrolens*, while the stability of plasmids from *Pseudomonas fluorescens*, *Pseudomonas chlororaphis* and *Pseudomonas aeruginosa*, is predominantly caused by genome of recipient strains. The maximal stability was detected on both selective and non-selective media in recipient cells of *Pseudomonas aeruginosa*.

Arginase isoforms activity and lipid peroxidation processes in the regions of the corticolimbic system in dexamphetamine-induced bipolar disorder

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Oxidative stress, including lipid peroxidation (LPO) processes is known to be involved in the pathophysiology of bipolar disorder (BD), as well as major depressive disorder and schizophrenia. Recent findings show that oxidative stress can trigger both expression and activity of arginase isoforms, which can be differentially implicated in the pathophysiology of BD, since ornithine produced by cytoplasmic A1 is involved in the proline or glutamate synthesis, whereas ornithine synthesized by mitochondrial A2, is involved in polyamine production. However, the role of arginase isoforms in BD has not been sufficiently investigated yet. Therefore, we studied BD-induced changes in the activity of arginase isoforms, as well as lipid peroxidation processes in the brain corticolimbic system regions responsible for emotions and affective memory (prefrontal cortex (PFC), striatum (ST), hippocampus (HC) and hypothalamus (HT)). We used experimental (rat) model of BD induced by escalating doses of dexamphetamine, withdrawal of which was accompanied by depressive-like behavior of rats, which attenuated in the second week and turned to remission-like phase in the third week. Alterations in gut resident microbiota were observed, namely the number of lactobacilli, bifidobacteria and *E. coli* decreased, its lactose-negative strains appeared, and the number of *Candida albicans* and the pathogenic *Staphylococcus aureus* increased significantly. Moreover, microbial translocation into the blood and brain occurred, and these changes were sustained but less pronounced in the remission-like phase. Notably, *C. albicans* is proved to be involved in depression. After dexamphetamine withdrawal, both A1 and A2 activity doubled in PFC, while in HC there was a predominant activation of A2, which was twice as high as A1, indicating a preferential synthesis of polyamines that trigger the mechanisms of cellular antioxidant defense, as well as proliferation processes. Both arginase isoforms, limiting the subcellular concentration of L-arginine, can reduce the level of tetrahydrobiopterin, which is the main cofactor of aromatic amino acid hydroxylases, and in this way suppress monoamine neurotransmission, leading to depression. In addition, arginase

isoforms can stimulate free radical oxidation processes, including LPO, there through also involving BD pathophysiology. After dexamphetamine withdrawal, a simultaneous increase in the level of malondialdehyde (LPO stable product and marker of oxidative stress) was observed in the regions of the corticolimbic system, namely, three times in PFC and HT, two times in ST and HC. Arginase isoforms activity and malondialdehyde level temporarily decreased within two weeks after dexamphetamine withdrawal, and three weeks later stimulated remission-like phase. We revealed this activation of arginases and LPO in BP remission for the first time, and these processes may precede BD recurrence. In summary, we identified a region-specific activation of arginase isoforms and LPO processes in the corticolimbic system in different phases of BD, which requires further study to determine the possibility of using arginase isoforms as therapeutic targets in BD.

Estimation of the effects of Ca and Mg ions on antifungal activity of lactic acid bacteria associations

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The shortening of various pathogenic fungi (molds and yeasts) growth in food manufacturing and storage has the principal significance. Fungi produce allergenic spores and mycotoxins causing essential health hazards and huge economical lesions. This problem becomes more and more urgent due to a small number of effective and natural antifungal preparations. It is known that lactic acid bacteria (LAB) have significant antibacterial and probably antifungal activity. That is why the study of antifungal activity of different LAB associations, as well as estimation of the metal ions (Ca^{2+}, Mg^{2+}) role in synthesis of antifungal substances is a new and very actual way for solution of this task. Thereby, the aim of this work was the analysis and estimation of the metal ions effect on antifungal activity of different LAB associations. Primary screening of LAB showed that the most active strains were *Lactobacillus rhamnosus* MDC9661, *Lactobacillus delbrueckii* subsp. *bulgaricus* RIN-2003-Ls, *L. delbrueckii* subsp. *lactis* MDC9632, *L. delbrueckii* subsp. *bulgaricus* MDC9633, *Streptococcus thermophilus* VKPM B-3809, *Enterococcus faecium* INR-2010-Tsov-G-St. Consequently, it was interesting to investigate the effect of divalent cations of Ca and Mg, as well as that of their combined mixture on antifungal activity of 15 different LAB associations. As a source of Ca and Mg ions $CaCl_2$ and $MgCl_2$ were used from 5 to 12 mM. The optimal concentrations of metal ions for antifungal activity of associations were around 10-11 mM. The results of these investigations displayed the following findings. The addition of Ca^{2+} on the growth medium of LAB significantly increased antifungal activity of some associations. The growth of most fungi was suppressed by the mixes including different combinations of MDC9661, RIN-2003-Ls, MDC9632, MDC9633 and VKPM B-3809. Most LAB associations without addition of ions could only inhibit the growth of *Fusarium oxysporum* and *Cladosporium herbarum*, while the supplementation of Ca^{2+} stimulated their antifungal effect also against *Penicillium aurantioviolaceum*, *Penicillium* spp., *Trichoderma viride*, *Geotrichum candidum* and *Aspergillus flavus*. It is also interesting to

mention that Ca ions in some cases displayed a negative effect on antifungal activity. Particularly, the mixture of MDC9661 and INR-2010-Tsov-G-St inhibited the growth of all tested molds, with the exception of *A. flavus*, but in the presence of Ca^{2+} it kept its activity only against *F. oxysporum*. Mg ions induced the antifungal activity of most LAB associations. In particular, various combinations of RIN-2003-Ls, MDC9632, MDC9633 and INR-2010-Tsov-G-St didn't show any inhibitory activity against all tested molds and only elongated the spore germination of *Cl. herbarum* in native conditions, whereas the addition of Mg^{2+} totally induced the antifungal activity of mentioned associations. Finally, the addition of a combined mixture of Ca and Mg ions essentially increased the antifungal effect of most associations. Interestingly, the natural LAB mixes of VKPM B-3809, MDC9632 and MDC9633 couldn't suppress the growth of any tested mold, however the supplementation of ions combination revealed their antifungal effect against all kinds of molds. The fact of substantial stimulation of the antifungal effect of most LAB associations by metal ions can serve a basis for creation of new effective antifungal preparations by the supplementation of ions combined mixture.

Approaches to studying the activity and stability of intracellular recombinant aminoacylase of *Escherichia coli*

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Aminoacylases, carrying out enantioselective hydrolysis of N-acyl-derivatives of DL-amino acids are widely used in industrial production of L- and D-amino acids. The aim of this work was development of approaches for studying the activity and stability of intracellular recombinant aminoacylase of *Escherichia coli* strain LGE36. Enzymes are exposed to 3 types of effects: denaturation (by: pH, temperature, organic solvents); proteolysis, inactivation of catalytic center. To solve the problem of proteolytic inactivation of intracellular aminoacylase of *Escherichia coli* we used mild conditions of enzyme purification, organic solvents were excluded. In the stage of obtaining the cell biomass the inhibitor of serine protease – phenylmethylsulfonyl fluoride (PMSF) was added at 1 mM final concentration. The complete inactivation of intracellular recombinant aminoacylase of *Escherichia coli* by parachlor mercury benzoate indicates the existence of free SH-groups, which are essential for enzyme activity. To protect the enzyme SH-groups, the sulfhydryl reagents were used, such as dithiothreitol (DTT), glutathione, ö-mercaptoethanol. The results indicate that only DTT, even in the case of similar redox potential compared with other reducing compounds, provides high activity and stability of the studied enzyme. The stability of intracellular recombinant aminoacylase of *Escherichia coli* in the presence of 1 mM DTT is enhanced more than 4 times, whereas the activity is enhanced 3 times, compared with control. It was shown as well, that ions of Co^{2+} at concentration of 0.1 mM increased the enzyme stability 3 times, and the activity – 10 times. So, it can be concluded, that using of DTT, PMSF and ions of Co^{2+} in strictly defined amounts, allows to obtain active and stable intracellular recombinant aminoacylase of *Escherichia coli* strain LGE36.

A bioinformatic human disease modeling based on genomic TF networks between ZIKA virus and human genomes

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Zika virus (ZIKV) is an arbovirus belonging to the *Flaviviridae* family, genus *Flavivirus*, such as Yellow Fever, Dengue and West Nile Virus. ZIKV infection was characterized by causing a mild disease presented with fever, headache, rash, arthralgia, and conjunctivitis; in some cases neurological and autoimmune complications may occur with signs attributable to Guillain-Barré syndrome and microcephaly. The recent scientific data bring evidence regarding possible complications for the fetus in association with ZIKV infection, in particular related to ZikaSPH2015 strain, when infection is contracted in the first trimester of pregnancy period. Transmission occurs through direct infection, blood products, saliva, urine and breast milk, sexual contact as well as via maternal-fetal if the virus is present in the placenta. Herein we reported an analysis of the entire genome of ZikaSPH2015 strain, performer to identify the occurrence of specific motifs able to bind and therefore subtract human transcription factors. As indicated in our results, the subtraction of these factors leads to an haploinsufficiency able to explain the complex symptomatology and complications for the fetus affected by ZIKA virus. The functional data derived from this approach could be extremely useful to identify specific clinical markers of risk profiling and therapeutic treatment.

A carbon source dependence of the antibacterial activity of lactic acid bacteria isolated from the intestinal tract of Armenian honeybees

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Lactic acid bacteria (LAB) are a widespread type of bacteria, which are representatives of gastrointestinal tract (GUT) microbiome of various organisms. Honey bees are the most important pollinating insect worldwide, as well as honey producers. The ecological diversity in Armenia led to creation of unique conditions for development of extraordinary bacterial associations demonstrating different properties. The microorganisms which are included in these associations arouse great biotechnological interest because of their metabolism. The bees feed on honeydew, which is composed of different carbohydrates, so, the investigation of the different carbohydrates effects on the growth and antibacterial activity of these strains is very important. On the other hand, there is an up-to-date direction of a new drug design connected with complex substances where the probiotics are combined with prebiotics. The non-microbial substances which improve the growth of probiotic microbes are known as prebiotics. The juice of Jerusalem artichoke can be used as a good source of different carbohydrates, especially of the widespread prebiotic inulin, while ashilajeet (mumijo) can be a good source of various minerals. The antibacterial activity was investigated by the well diffusion method. In modified media the glucose was changed with 1% of different carbohydrates, such as sucrose, fructose, lactose, mannose, etc. The LAB isolated from gastro-intestinal tract of bees have antibacterial activity mainly against Gram positive bacteria. The antibacterial activity of LAB depends on the media composition, more precisely, on the source of carbon. The replacement of glucose with sucrose or maltose leads to inhibition of antibacterial activity against Gram positive test-organisms, but crucially increases it in case of Gram negative bacteria. The addition of 1% Jerusalem artichoke (*Helianthus tuberosus L.*) juice or 5% and 15% shilajeet to the media yields the inhibition of antibacterial activity as well. Thus, despite the fact that studied LAB were isolated from the GUT of honeybees, the glucose replacement with other carbon sources leads to the decrease of antibacterial activity. On the other hand, the use of these microorganisms can lead to the creation of new functional foods with low glucose content.

Studies of the productivity and antioxidant activity of carotenoids of *Thermus scotoductus* K1 under different growth conditions

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Carotenoids, yellow to red fat-soluble pigments found in plants, algae, fungi and bacteria, are widely used in the production of colorants, cosmetics, food, antioxidant and anti-cancer preparations. In contrast to eukaryotes, bacteria produce carotenoids with greater number of carbon atoms, conjugated by double bonds and hydroxyl groups, which all contribute to their great antioxidant capacity. Carotenoids are particularly important in adaptation mechanisms to extreme conditions. Thermophilic bacteria from the Deinococcus-Thermus phylum actively produce carotenoids involved in mechanisms of protection against heat stress. The aim of the present study was to investigate the effect of cultivation conditions (temperature, pH, aeration, and lighting) on biomass and general carotenoids productivity of *Thermus scotoductus* K1 isolated from the geothermal spring of Karvachar, Nagorno-Karabakh. The free radical scavenging activity of carotenoid extracts was determined as well. The carotenoids were exhaustively extracted by maceration of dry biomass with methanol for 24 hours on magnetic stirrer, and then concentrated under reduced pressure in a rotary evaporator. Antioxidant property of carotenoid extract was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging using ascorbic acid as positive control. The maximum production of biomass of *T. scotoductus* K1 (0.3 g/L) and specific productivity of carotenoids (1.3 mg/g) was obtained at 65 °C, pH 8, in aerobic conditions. Slightly alkaline pH (8-9) values had a positive effect on biomass production and yield of carotenoids in both dark and lighting conditions. Although the specific productivity of carotenoids is not dependent on the lighting conditions, the lighting affects the composition of the carotenoids. The main carotenoids of *T. scotoductus* K1 were *thermozeaxanthins*. In the tested conditions, changes in the levels of the variables influenced the biomass and carotenoid production, although they did not influence the carotenoid profile. Carotenoid extracts from *T. scotoductus* K1 showed good DPPH radical scavenging activity. It was shown that carotenoids extracted from biomass grown in light and dark conditions exhibited 62% and 82% of antioxidant activity, respectively. The results of

this study provide a better understanding of the effect of cultivation conditions of a thermophile bacterium, *T. scotoductus* K1, on biomass and carotenoid amounts.

This work was supported by grants from the Eurasia programme of the Norwegian Center for International Cooperation in Education CPEA-LT-2016/10095) and partially supported by the RA MES State Committee of Science, in the frames of the research project №15T-1F399.

Antimicrobial activity of biosynthesized metallic nanoparticles

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The synthesis of nanostructured materials can be considered a research field of high importance, especially over the past decade, due to the unique properties that make these materials applicable in different fields of science and technology. Metallic nanoparticles gained significant interest due to the possibility to obtain them through biological means, among other techniques. In this regard, it has been shown that plant extracts and microorganisms, used in green synthesis, have the capability to produce nanoparticles without the use of harsh, toxic and expensive chemicals commonly used in conventional physical and chemical processes. Therefore, the reducing agents involve various water-soluble plant metabolites, namely, alkaloids, phenolic compounds, terpenoids, flavonoids, saponins, steroids, tannins and other nutritional compounds and co-enzymes, while polysaccharides, proteins and lipids act as capping agents in the synthesis process [1]. Silver nanoparticles are some of the most investigated metallic nanoparticles, due to their recognized antimicrobial properties. These silver nanoparticles can exert their effect on microbial cells by generating membrane damage, oxidative stress, and injury to proteins and DNA. This study aims to summarize the emerging efforts to address current challenges and solutions in the treatment of infectious diseases, particularly through the use of silver nanoparticles biosynthesized via microbes and plants pathways.

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Tartaric acid new derivatives antibacterial activity and biodegradation by non-pathogenic soil strains of *Pseudomonas chlororaphis*

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Multidrug resistance of bacteria is the one of the most important problems of modern medicine and ecology. Different species of Pseudomonas genus are well-known by their multidrug resistance. One of the ways to combat the antimicrobial resistance is the search for alternatives to classical antibiotic therapy, using natural plant extracts or synthetic compounds synthesized on the basis of natural products. Tartaric acid is the one of the most common compounds in nature. This aldaric acid is commonly found in cells of plant. It is well-known as an antibacterial agent, broadly used in beverage and food production industry. The main aim of current research was the elaboration of a new safe, economically effective compound with high antibacterial properties, based on natural tartaric acid. 4 New derivatives of tartaric acid were synthesized based on natural cream of tartar (the waste product of wine industry) by the simple 2 stage technology. Benzyl imide, cyclohexyl imide, benzyl mono amino salt and cyclohexyl mono amino salt of tartaric acid have demonstrated antibacterial activity in respect of some Gram-negative and Gram-positive bacteria including pan-drug and multidrug resistant strains of *Pseudomonas*.

It was also shown that some strains of *Pseudomonas chlororaphis* are able to degrade these compounds. The resistance to these compounds can't be transferred to the sensitive strains by plasmid transformation.

Thus, benzyl imide, cyclohexyl imide, benzyl mono amino salt and cyclohexyl mono amino salt of tartaric acid may be recommended as antimicrobials for detailed study of their potential.

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Study of the *Pseudomonas* genes coding polyphenol oxidases

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Pseudomonas species are very common inhabitants of wet surfaces world around. They are well-known by their resistance to various chemical and physical stress agents, including synthetic xenobiotics and antibiotics. Drug resistance creates many problems in the therapy of infections caused by *Pseudomonas* in clinics. Simultaneously, different species of *Pseudomonas* possess a wide spectrum of enzymes participating in biodegradation. The cyclic and aromatic organic compounds are very dangerous ecological pollutants, which are known as toxic for animal and human organisms inducing cancer and other diseases. The study of their biodegradation by enzymes produced by soil microorganisms is important from scientific and practical point of view. The main aim of current research was to compare Polyphenol oxidase activities in 6 different species and 3 subspecies of soil *Pseudomonas* strains, using microbiological, biochemical and genetic methods. As a result the different types of polyphenol oxidases were identified. According to the obtained data, polyphenol oxidase activities of studied strains were not transferred by plasmids, which indicated chromosomal localization of genes encoding those enzymes. In 20% of studied strains polyphenol oxidase activity was coupled with resistance to tetracycline.

This research is supported by the research grant No microbio-5133, from the Armenian National Science and Education Fund (ANSEF) based in New York, USA and the RA MES State Committee of Science, in frames of the research project № 18T-2I036.

Isolation and identification of novel antibiotic–producing soil bacteria from extreme econiches

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One of the most actual problems in modern biomedical science is the investigation of new producers of biologically active substances which inhibit growth of pathogenic microorganisms. Traditional producers of natural antibiotics were isolated from different medicinal plants' phyllosphere. Our objective was screening and isolating, identifying new members of beneficial antibiotic procedures among halophyte rhizosphere which grow in salinated soils. These extreme ecosystems' microbial communities are more resistant and survive harsh environmental conditions which propose studying their antagonistic features.

Soil samples for microbiological analyses were collected from the plant rhizosphere *Suaeda salsa* (L.), growing on saline soils of the drying Aral Sea. For isolating antibiotic producers were taken elective organic Gauze media with 3.0 % NaCl (gr/L): tryptone – 3.0; NaCl – 30; peptone – 5.0; agar – 20.0; glucose – 10.0; distilled water – 1000 ml, pH –7.2 (Kulikova 2017). Obtaining enrichment and pure cultures were performed according to standard methods (Yemtsev, Mishustin 2018) for 4 days at 37 °C. Molecular–biological identification (extract DNA, amplification, PCR and sequencing 16S rRNA) was carried out by lab protocol.

After enrichment of cultures two isolated differed, which in the next stages of culturing were marked as two pure strains. The results of sequencing 16S rRNA partial gene showed that isolated strains affiliated to genus *Micrococcus* and *Halomonas*. *Micrococcus* sp. is a gram-positive tetracoccus shaped, obligately aerobic, mesophyll saprophyte bacterium. Several species synthesize antibacterial substances such as C-1, C-3, C-5, C-7 which are bacteriocins (Gorbatko, 2009). *Halomonas* sp. is a gram-negative, rod-shaped, halophilic proteobacteria. We suppose that results in our research on screening and isolating new representatives of microorganisms with antibacterial properties may be prospective for developing novel antibacterial biologically active compounds.

Moderately halophile nitrogen-fixing bacteria of salinated arid soils

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Polyextremophile ecosystems are considered to be those econiches in which the biotechnological potential of this microbiome are not explored fully. Several key biochemical reactions (methanogenesis, sulfate-reducing, anammox) are processed due to extremophile microorganisms. Biogeochemical cycling of nitrogen in the biosphere is one of the key processes which during the globally changing climate require deep analysis for adaptation the agriculture to global climate change. Our purpose from performing this research was screening, isolation and identification, including identifying the presence of nifH gene in halotolerant and halophile bacteria from salinated arid soils of Uzbekistan and Syria.

Enrichment cultures and isolating pure cultures and cultivation were carried out in modified Ashby media including saccharose – 20,0; K_2HPO_4 – 0,2; $MgSO_4$ – 0,2; NaCl – 50; K_2SO_4 – 0,1; $CaCO_3$ – 5,0 for four days at 37 °C; molecular identification of selected strains was provided by sequencing the 16S rRNA gene. Detection of the nifH gene responsible for the nitrogen-fixing activity was performed by the method of real-time PCR. Results and discussion. The results show that after the enrichment in modified nitrogen-free media containing 5% NaCl the strain isolated from solonetz soil can grow on it. The sequencing 16S rRNA gene showed that strains related to the genus *Halomonas*, *Virgibacillus*, *Halobacillus* and *Gracilibacillus*.

We propose that isolated moderately halophile bacteria *Halomonas*, *Virgibacillus*, *Halobacillus* and *Gracilibacillus* are free-living nitrogen bacteria from arid salinated soils of Uzbekistan and Syria. The research on isolating and studying free nitrogen-fixing bacteria, especially with halophilic nitrogen-fixing bacteria of arid salinated soils (complex degraded), provides theoretical concept and knowledge thus offering future solutions to evaluate and develop some potential biotechnological applications for promoting plant growth and improving the biological activity of degraded agricultural lands.

Photodynamic inactivation of methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus*

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Increasing antibiotic resistance among pathogenic bacteria has forced researchers to find alternative therapeutic options against which the bacteria will not be easily able to develop resistance. Photodynamic inactivation (PDI) of microorganisms is one of the most promising alternative therapeutic approaches. The main advantage of PDI is its multi-target action. This therapeutic approach utilizes visible light, a non-toxic photosensitizer (PS) and molecular oxygen to create highly toxic reactive oxygen species (ROS). These species, such as singlet oxygen and free radicals are able to irreversibly alter vital components of cells resulting in oxidative lethal damage. This study was aimed at the investigation of differences in response to tetracationic Zn-mesotetra-[4-N-(2`-butyl)pyridyl]porphyrin (Zn-TBut4PyP)-mediated photo-inactivation between methicillin-resistant *Staphylococcus aureus* (MRSA), one of the most common causes of wound infections in the world, and methicillin-sensitive Staphylococcus aureus (MSSA) strains *in vitro* conditions. Samples of bacterial cell suspensions (108 CFU ml−1) with appropriate concentrations of Zn-TBut4PyP were irradiated with a power of 30 mW cm−2 for 30 min and then viability of bacterial cells was assessed after serial dilutions by a colony-forming assay. It was showed that both strains were equally susceptible to photodynamic inactivation when the appropriate concentration of photosensitizer was used. Incubation with 0.5 μg/ml of Zn-TBut4PyP followed by irradiation yielded a 99.9 % decrease in the viable numbers of MRSA and MSSA strains. In experiments we tested the possible development of microbial resistance to PDI by Zn-TBut4PyP. It was shown that the both strains after 10 successive photodynamic procedures with Zn-TBut4PyP did not result in any resistant mutants against the PDI. This suggests that PDI via Zn-TBut4PyP can be considered as a convenient innovative strategy for treating localized MRSA infections.

Keywords: photodynamic inactivation, photosensitizer, methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *Staphylococcus aureus*

Characterization of the growth of salt tolerant nodule bacteria from Armenian soils

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About 36.5% of the soil in the Republic of Armenia is not cultivated and not purposely used because of salinity, which is one of the main obstacles for the development of agriculture. Salinity has a detrimental effect on the activity of rhizobia, for example, it reduces the number of rhizobia cells in the rhizosphere and limits root infection, which is accompanied by inhibition of development and a deterioration in the nitrogen-fixing activity of nodules. Some types of symbiotic nitrogen-fixing bacteria exhibit high salt tolerance [1, 2, 3]. Our aim was to evaluate the salt tolerance of the species *Sinorhizobium meliloti* and *Bradyrhizobium japonicum*, which were stored in the collection of the laboratory of nitrogen-fixing microorganisms. Various molar concentrations of NaCl, $MgSO_4$, $NaHCO_3$ and Na_2CO_3 were added to the medium (YEMA) to determine the salt tolerance of the 10 strains. The growth of nodule bacteria was evaluated on a three-point scale. According to the results of the experiments, it was found that the strains of the studied nodule bacteria of alfalfa proved to be the most tolerant to molar salt concentrations. Two strains of *S. meliloti* showed abundant growth (+++) at a NaCl concentration of 0.02-0.4M in the medium. The remaining three strains gave (++) growth at a concentration of 0.02-0.5M at a $MgSO_4$ concentration from 0.025-0.2M, growth is (+++), with a $MgSO_4$ concentration from 0.3-0.5M (++). Molar solutions of Na_2CO_3 and $NaHCO_3$ had the most inhibitory effect on the growth of soybean strains compared to strains of *Sinorhizobium meliloti*-with growth (++) at a concentration of $NaHCO_3$ from 0.01-0.5M. At a content of 0.005-0.1M Na_2CO_3 in the medium, all strains showed very poor growth. Molar solutions of Na_2CO_3 exerted the most depressing effect on the growth of soybean strains. Only one soybean strain showed the most stable growth, it grew slightly (+) at a concentration of up to 0.5M Na_2CO_3 in the medium. Soybean strains did not grow well at a concentration of 0.5M NaCl in the medium. Three soybean strains grew on a medium containing 0.2M $MgSO_4$ (+++) and at a concentration of 0.5M $MgSO_4$ growth was slight (+). Among the strains, one soybean strain was distinguished, which revealed stability with poor growth (+) at a concentration of up to 0.5M Na_2CO_3 in the medium. All strains of the studied alfalfa nodule bacteria used D-ribose,

L-arabinose, D-xylose, D-maltose, D-lactose, D-mannose, D-galactose, glucose, fructose as a carbon source and did not absorb dulcite. The strains used ammonium salts for growth better than nitrates and nitritas as sources of nitrogen. *Bradyrhizobium japonicum* strains did not metabolize sucrose. All strains did not grow on peptone. Strains of nodule bacteria of alfalfa and soybeans, which grow under severe salt stress conditions were selected as a result of this study.

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Application of aminotransferases in asymmetric synthesis of amino acids

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Pyridoxal-5'-phosphate (PLP) dependent enzymes catalyze different reactions connected to amino acid metabolism. These enzymes can be incorporated in three main families (alfa, betta and gamma), on the basis of amino acid sequence similarities. The study of catalyzed reactions indicated that the family affiliation is mainly correlated with regiospecificity of corresponding enzymes. In the biggest alfa-family of PLP-dependent enzymes the covalent change takes place with the α-carbon atom, carrying amino group and the imino-bond is formed with the PLP of enzyme in the course of the reaction. It comprises glycine hydroxymethyltransferase, glycine C-acetyltransferase, 5-aminulevulinate synthase, 8-amino-7-oxononanoate synthase, all aminotransferases, a number of other enzymes relatively closely related with the aminotransferases and very likely a certain group of amino acid decarboxylases as well as tryptophanase and tyrosine phenol-lyase which, however, catalyze betta-elimination reactions.

The aminotransferases could be divided into four subgroups on the basis of their mutual structural relatedness. Subgroup I comprises aspartate, alanine, tyrosine, histidinol-phosphate, and phenylalanine aminotransferases; subgroup II acetylornithine, ornithine, omega-amino acid, 4-aminobutyrate and diaminopelargonate aminotransferases; subgroup III D-alanine and branched-chain amino acid aminotransferases, and subgroup IV serine and phosphoserine aminotransferases. Apparently, the aminotransferases constitute a group of homologous proteins which diverged into subgroups and, with some exceptions, into substrate-specific individual enzymes already in the universal ancestor cell. As of August 2019, in the list of Enzymes Nomenclature (EC) of the International Union of Biochemists and Molecular Biologists the aminotransferases are presented by 115 members (EC 2.6.1.1 – EC 2.6.1.115). But for various reasons - removal, relocation to another group, association with other types of aminotransferases, etc., 7 of them have been removed, and in fact these enzymes are now represented by 108 members. The aminotransferases have broad substrate specificity and absolute stereo-specificity, enabling the same biosensor to be used in the stereoselective synthesis of several compounds at the same time. In the presented report

some physicochemical and catalytic properties of valine-pyruvate, alanine, aspartate and aromatic aminotransferases, as well the possibilities of using some aminotransferases in stereoselective production of amino acids, are discussed.

Heavy metal resistance of *Arthrobacter oryzaea* VL55 and *Pseudomonas* sp. AI2 strains

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Metal-rich natural and artificial habitats are extreme environments for the development and evolution of unique metal tolerant microbial communities, which could have important application in bioremediation of metal contaminated environments. The goal of presented work was the study of heavy metal resistance of the *Arthrobacter oryzaea* VL55 and *Pseudomonas* sp. AI2 strains, which were isolated from Shamlugh copper mine and Artsvanik tailing, correspondingly. The strains demonstrated high resistance to the Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} ions. In order to optimize growth media, strains were cultivated in VL55, R2A and NB media with pH 5.5, 6.0 and 7.5, correspondingly. The results showed that *A. oryzae* VL55 strain exhibited best growth in R2A media in all pH. The high resistance of *A. oryzae* VL55 strain was observed against Zn^{2+} (MTC is 4.8 and 4.9 mM in VL55 (pH 5.5) and R2A (pH 6.0) medium, correspondingly) and Ni^{2+} ions (MTC is 5.9 mM in R2A (pH 6.0) medium). *Pseudomonas* sp. AI2 strain exhibited high resistance to Cu^{2+} in NB medium with pH 7.5 (MTC is 2.8 mM). The effect of different concentrations of separate, as well as combinations of Cu^{2+}, Ni^{2+} and Zn^{2+} ions on bacterial growth in appropriate media was observed as well. The results demonstrated that although *A. oryzae* VL55 growth was inhibited by Ni^{2+} ions (up to 3mM) the stationary growth phase of the strain started 10 hours later compared with control. The bacterial growth was inhibited by combined Cu^{2+}, Ni^{2+} and Zn^{2+} ions (1.0, 1.0 and 2.0 mM, correspondingly). The study of the effect of different concentrations of Cu^{2+} ions on *Pseudomonas* sp. AI2 strain growth showed that low concentration of Cu^{2+} (0.2-0.4 mM) stimulated bacterial grow though in case of high concentration the inhibition of bacterial growth was observed. The results are highlighting the importance of *A. oryzae* VL55 and *Pseudomonas* sp. AI2 strains as new bioremediation tools.

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Effects of combined inoculation of *Bambara Groundnut* (*Vigna subterranean* L. Verdc.) with *Glomus Mossea* and *Bradyhrhizobium Japonicum* on nitrogen and phosphorous uptake in shoot, plant biomass and leaf chlorophyll

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The response of *Bambara groundnut* to coinoculation with *Abuscular mychorrhizal* fungi (G. Mossea) and *Bradhyrhizobium japonicum* (strain USDA110) with regard to leaf chlorophyll, percentage shoot nitrogen and phosphorus, nodule weight and plant biomass was studied. Bambara plants were grown under screen house conditions in pots. Plants were inoculated with 1ml of *B. japonicum* USDA 110 strain (10^9 cfu /ml), Mychorrizal was applied to the plants 10g, and 20g (90 spores/g) and water was applied at 10ml, 20 ml and 50ml every other day. The obtained results showed that dual inoculation activity was able to improve both nitrogen and phosphorus in plant shoot but did not improve biomass and leaf chlorophyll when compared with plants subjected to single inoculation with only *G. Mossea* and only *B. japonicum.* More Nitrogen and Phosphorus were retained in the shoot of plants coinoculated with *B. japonicum* and 20g *G. Mossea* when given 50ml of water and also had higher biomass. Leaf chlorophyll reduced in plants as flowering approached. *B. japonicum* was able to positively influence and establish symbiosis with *G.Mossea* and synergistically effectively act as "mycorrhiza helper bacteria" (MHB) when both were coinoculated in Bambara plant.

Agrobacterium sp. as nitrogen-fixing bacteria: effect of osmo-resistant strains on the growth of plants in saline soils of Armenia

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In a complex set of studies of soil processes, biological approaches are of particular significance, and therefore one of the most important aspects is the study of the biological activity of soils, since it makes it possible to know the direction and intensity of the biological processes occurring in them. Chemical fertilizers, herbicides and pesticides used in agriculture, after prolonged use, leave negative consequences for soil quality, up to salinization and degradation [1, 2]. An equally important cause of salinization and soil degradation is an increase in groundwater levels and lack of a drainage system. The combination of all these factors ultimately led to severe salinization of soils in many villages of the Ararat and Armavir regions of Armenia. In order to correct the situation and keep it under control, it is necessary to minimize the use of environmentally unreasonable chemicals and, taking into account modern world experience, use environmentally friendly bio-fertilizers obtained by biotechnological methods based on nitrogen-fixing microorganisms that are resistant to high salt concentrations.

Previously, a collection of nitrogen-fixing bacteria was isolated from saline soils of the Armavir and Ararat regions, the morpho-physiological and biochemical properties of the selected cultures were studied, and their nitrogen-fixing activity was determined [3]. Bacteria with the highest nitrogen-fixing activity were identified by sequencing (Macrogen Inc., South Korea). According to the identification results, two of the most active strains (M-1 and E-2) were assigned to *Agrobacterium* sp. Based on these strains, mutants resistant to high salt concentrations or, in other words, osmo-resistant strains (Osm-r) were obtained. To assess the effect of Osm-r nitrogen-fixing strains on the growth and development of beans in saline soils, a series of specially performed experiments were carried out. The results of the experiments have shown that the use of these cultures has a noticeable stimulating effect (about 30-40%) on the growth and development of the tested plants under high osmotic pressure. Based on the data obtained, it can be concluded that osmo-

resistant (Osm-r) nitrogen-fixing strains can serve as an effective basis for creating a new biofertilizer that will be highly active in saline soils.

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Microbiota Tamdymkul of geothermal springs in Tajikistan

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Thermophilic bacteria are less studied but important group of microorganisms due to their ability to produce industrial enzymes.

In this study, thermophilic bacteria were isolated from hot spring of Tamdymkul, Tajikistan. A bacterium that could tolerate high temperatures was characterized by morphology, biochemistry and sequencing of its 16S rRNA gene. The isolate was screened for amylolytic, proteolytic and lipolytic activities. Phylogenetic affiliations and G+C content of the isolate was studied. Spring is located at 39°157 ″N, 071°13,130 ″E, at 2000-2135 m above sea level, has 88 °C temperature, pH 7.4, conductivity 1019 μS/cm.

API 50 CHB strips were used to identify isolates. DNAs were extracted from all isolates and their amplicons of 16S rRNA genes were obtained by PCR using universal primers. All amplicons were successfully sequenced and obtained sequences aligned with sequences available in Gen Bank by BLASTn tool.

As a result, the majority of sequences were shared 94-99 % similarities with representatives of genus *Geobacillus* (*G. thermoglucosidasius*, *G. caldoxylosilyticus*, *G. stearothermophilus*).

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Genome analysis of *Bacillus subtilis* VA 44

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Based upon our preliminary results in the screening of strains with active antibacterial and antifungal properties, the strain *B. subtilis* VA 44 is studied in detail as a potential industrial probiotic strain. Genome sequencing of the strain VA 44 was carried out using the Illumina HiSeq platform to implement genome mining for preliminarily characterization of its bioactive secondary metabolites and evaluation of its antimicrobial activity. Core genome phylogeny showed that the strain VA 44 was located within the *Bacillus subtilis* subsp. *inaquosorum clade*. Strains of this clade are known for their antibacterial, antifungal, and probiotic activities. The strain VA 44 is the most closely related both to *B. subtilis* subsp. *inaquosorum* strain DE111, a commercially produced human probiotic, and to antifungal strain HU Biol-II. The sequenced genome of *B. subtilis* VA 44 contains the genes responsible for the synthesis of peptide and lipopeptide antibiotics, and bacteriocins (surfactin, fengycin, etc.). Various aspects of comparative genomics of the *Bacillus subtilis* subsp. *inaquosorum* strains are discussed regarding their remarkable biocontrol properties.

Acidophilic bacteria: biodiversity, metabolism, ecology and application

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Acidophilic bacteria are extremophiles that inhabit extremely acidic environments with pH value below 3 and moderately acidic environments with pH value between 3 and 5. Natural acidic environments are relatively rare; most of them are located in parts of the world with an intense volcanic activity where sulfur-rich geothermal springs occur. These springs named "solfatara" are acidic due to the activity of sulfur-oxidizing acidophilic bacteria. Bacterial sulfur oxidation produces sulfuric acid, which makes these habitats extremely acidic. Another natural acidic environment is Rio Tinto in Spain. This river flows through pyritic belt and acidity of the water as well as its deep red color is the consequence of pyrite (FeS_2) oxidation by acidophilic iron-oxidizing bacteria. Unlike natural acidic environments, artificial acidic environments are widespread and associated with the mining of metals and coal. Mining activities lead to exposure of sulfide minerals (mostly pyrite) that are deep underground to oxygen and water. Chemical oxidation of pyrite generates ferrous iron, sulfur compounds and acidity, which are conditions where acidophilic microorganisms thrive. Metabolic activity of acidophilic microorganisms significantly accelerates the dissolution of pyrite and other metal sulfides, resulting in the formation of acid mine drainage (AMD). Acidic mine waters originate in underground and open-pit mines, on the mining waste dumps, and in abandoned open-pit mines forming acidic lakes. The biodiversity of acidophilic bacteria is limited, but these microorganisms have diverse metabolic traits. Many acidophiles are chemolithoautotrophs that use iron and sulfur as a source of energy and CO_2 as a source of carbon; some acidophiles oxidize iron but use organic compounds as a source of carbon, and others are obligatory heterotrophs. Generalists like genus *Sulfobacillus* sp. can switch from heterotrophic to autotrophic metabolism. Most of the acidophilic bacteria can switch from aerobic to anaerobic metabolism. Hostile environments with extremely low pH can be full of life on a microscopic scale. These ecosystems are populated by acidophilic algae, fungi, protozoa and bacteria. The productivity of these ecosystems can be higher in comparison to freshwater ecosystems. The ability of acidophilic bacteria to oxidize iron, sulfur and sulfide minerals was applied in extractive metallurgy. Bacterially

assisted extraction of metals from ores and other mineral raw materials is named “bioleaching”. It is an important technology in copper production. The environmental impact of acidophilic bacteria and its application in mining and metallurgy led to intensive research of these microorganisms. The aim of this lecture is to outline biodiversity, metabolism, ecology and applications of these extremophiles.

Tolerance of isolated metal leaching bacteria to Mo^{6+}

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At present microorganisms more frequently occur with high concentration of metals in their habitats due to natural processes and human industrial activities. Many metal ions have a key role in physiology of cells. Some of them are incorporated into metalloproteins such as cytochromes, blue copper proteins and play an essential role in electron transport [1]. Transition metals act as electron carriers during redox reactions of electron transport chains to generate chemical energy [2, 3]. Metal ions also function as cofactors and confer catalytic potential to proteins [4]. However, at high concentrations heavy metals form complex compounds in the cell and can disrupt cell membrane, hinder cellular functions, and damage DNA [5]. To tolerate the presence of heavy metals (Cu, Zn, Fe, Co, Mn, Mo, etc.), microorganisms have evolved several mechanisms such as efflux, complexation or reduction of metal ions. Some of them can use metals as sources of energy or terminal electron acceptors in the oxygen limited conditions. Microorganisms that are resistant and grow on metal ions play an important role in their biogeochemical cycling. *Acidithiobacillus ferrooxidans*, *Leptospirilum ferrooxidans* and *L.ferriphilum* predominantly found in technogenic wastes (acid mine drainage, ore dumps and tailings) where high concentration of heavy metals occurs have been considered as the most important microorganisms for recovery of metals from primary and secondary raw materials. Their significance is due to the ability to use both iron and sulfur contained in raw materials as energy sources for growth. The study of tolerance of these microorganisms to high concentrations of metals is of great scientific and practical interest. It is triggered by the necessity of obtaining resistant strains that can be useful for biomining and bioremediation. The influence of molybdenum (Mo^{6+}) on the growth and iron oxidation by *Leptospirilum ferriphilum* CC and *Acidithiobacillus ferrooxidans* ZC isolated from bioleaching pulp of copper and zinc concentrates [6] was investigated. Experiments were performed in batch cultures in the concentration range of sodium molybdate (Na_2MoO_4) from 0.5 to 20 mM. It was shown that sodium molybdate (Mo) in concentration of 0.5mM completely inhibited the growth and iron oxidation in *L.ferriphilum* CC. The minimal inhibitory concentration of Mo^{6+} for *L.ferriphilum* CC was 0.1mM. On the contrary, *At. ferrooxidans*

ZC was able to grow and oxidize iron at concentrations of Mo^{6+} up to 5mM. Minimal inhibitory concentration of Mo for *At. ferrooxidans* ZC was 1mM. Thus, iron oxidation by *At. ferrooxidans* ZC for 28 h was inhibited by 34.5; 47.6 and 77.1 times in the presence of 1, 2 and 5mM of sodium molybdate, respectively. It was revealed that the extent of iron oxidation inhibition in *At. ferrooxidans* ZC directly depended on the age and amount of inoculum (initial bacterial cell number). Microorganisms' tolerance to heavy metal stress was much higher in logarithmic phase of the growth. The more inoculums, the less iron oxidation inhibition by sodium molybdate was observed. It is concluded that the increase of the amount of inoculum will allow in some ways to overcome the inhibitory effect of Mo. From data obtained it should be noted that bacterial cells during their growth form biofilms, which significantly increase the resistance of bacteria to heavy metal ions stress. Keywords: metal leaching bacteria, heavy metals, tolerance to molybdenum, minimal inhibitory concentrations.

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Biohydrometallurgical processing of Kajaran low-grade copper-molybdenum ores

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The treatment of low-grade ores which may be considered as an important source of base and precious metals requires the development of high-efficient approaches for metal extraction. The goal of the present work was to develop biohydrometallurgical approach based on microbiological leaching of Kajaran low-grade copper- molybdenum ores. Sulfide and oxidized ore samples containing 0.63 and 0.77% of copper, 1.81 and 1.55% of iron and 0.14 and 0.023% of molybdenum, respectively were used. The experiments were carried out in 250 ml flasks under shaking conditions. Indigenous and adapted consortia of "Kashen" and "Kavart" bacterial cultures were used for bioleaching of ore samples. The influence of particle size (PS), pulp density (PD), adaptation of culture on the process of copper bioleaching was studied. The obtained results showed that the highest efficiency of copper bioleaching for both sulfide and oxidized ore samples was observed at PS of 45μm and PD of 20%. Besides, "Kashen" culture showed higher activity of copper extraction from both tested ore samples at 20% of PD. "Kavart" culture was more active in bioleaching of oxidized ore samples at 10% of PD. In the next series of the experiment on bioleaching, "Kashen" and "Kavart" cultures adapted to the tested samples by several passagings in the presence of gradually increasing concentrations of ores were used. It was revealed that the use of adapted cultures allowed increasing the extent of copper extraction by about 5 times up to 91-94% and 98% from sulfide ore within 15 days at 10 and 20% of PD. In case of the oxidized ore, the extent of copper extraction by the mentioned adapted cultures was doubled, reaching 100% in 2-3 days. As shown from the obtained data, 9K medium was more effective for bacterial growth and copper extraction compared with Mac medium. At the next stage of the investigation, bioleaching of ore samples was carried out in glass columns (percolators) 35mm diameter and 220-230mm height using "Kashen" and "Kavart" microbial consortia. Glass columns were loaded with oxidized ore samples with PS varying in the range of 0.8-3.5 mm and 9K medium (1:1). Percolation was supplied by microcompressors. The experiments were performed in two stages. At the end of the first stage pregnant sulutions were replaced with fresh 9K medium. The obtained results showed that for 13 days of bioleaching 18

and 26-28% of copper was extracted in the absence and presence of microbial consortia “Kashen”, respectively. At the second stage additional 37-49% of copper was extracted by microbial consortia. Investigations showed that the column bioleaching was greatly affected by PS. Thus, the reduction of PS to 0.8-1.6 mm led to the increase of copper extraction from oxidized ores by about 3 times and reached 100% in 9-12 days compared with that of control without bacteria (84%). The results suggest that bacterial leaching can be an effective approach for the treatment of low-grade copper-molybdenum ores and is a promising method for copper extraction.

Keywords: sulfide and oxidized ores, bacterial consortia, copper extraction, particle sizes, pulp density, adaptation, column bioleaching

Photobleaching of photosensitizers solutions under different lighting conditions

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Photosensitizers (PS) used in photodynamic therapy of tumors (PDT) - porphyrins, chlorins and phthalocyanines - have different photostabilities. Therefore, research of photostability of these compounds is an important task. AIMS: The aim of our research is to study photobleaching of some PS under different lighting conditions. METHODS: The cationic porphyrins TOEt4PyP, TBut4PyP, Zn-TOEt4PyP, Zn-TBut4PyP, anionic chlorin e6, and neutral Al phthalocyanine were used as photosensitizers. Photobleaching of photosensitizers was carried out under: 1) irradiation with a tungsten lamp (fluence rate: 30 mW/cm^2) and 2) exposure to sunlight (fluence rate:70 mW/cm^2). Changes in absorption intensities were recorded spectrally using a spectrophotometer.

Photobleaching of all cationic porphyrins was insignificant under irradiation with a tungsten lamp, (absorption intensity of solutions illuminated up to 1 hour was at the level of 81-98.5%). Porphyrins were photobleached partially under the sunlight (absorption intensity of solutions decreased by 3% - 27%). Chlorin e6 absorption intensity decreased by 17.7% after irradiation with a tungsten lamp, and it significantly photobleached under the sunlight (absorption intensity of solutions decreased by 40.3%). There was no photobleaching for Al-phthalocyanine regardless of illumination mode.

Chlorin e6 exhibits partial photobleaching, this photosensitizer is recommended for use with strict protection from the light. Cationic porphyrins and the photosensitizer Al-phthalocyanine practically are not photobleached under both illumination modes: therefore, they can be used as photostable compounds for PDT.

Insecticidal preparations and plant growth stimulator based on melaninogenic *Bacillus thuringiensis* strains

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The pesticides used in agriculture and forestry are toxic not only for pests, but also for humans, animals, birds, fishes and even for useful insects and this can lead to hazardous environmental contamination. Currently the use of biological insecticides, which are completely free of specific drawbacks of chemical control and simultaneously have special target effect on pests, is the most efficient and environmentally safest measure for the protection of plants. Based on insecticidal *Bacillus thuringiensis* strains the obtained melanin-synthesizing mutants retain their insecticide activity. The technologies for obtaining both bacterial melanin (BM) and bioinsecticidal preparation on the basis of the obtained melaninogenic strains have been developed. The safety of BM and bioinsecticidal preparations to environment has been demonstrated. BM is used as a growth stimulator on various plants. In comparison with other applied growth stimulators BM surpasses them in a number of properties: it is water-soluble, rapidly decomposes in the soil, is efficient at very low concentrations and is low cost, which has become a prerequisite for its application as a growth stimulator on various plants. One of the characteristic signs of melanin is its photoprotective activity, which protects the spores and crystals of insecticide strains from damaging effects of UV-radiation and insolation. The experiments in laboratory and field conditions on various agricultural pests (cabbage and apple moths, potato beetle, etc.), as well as on several types of mosquitoes have shown that the insecticidal activity of the obtained melaninogenic mutants of *B. thuringiensis* distinctly increases as compared to the parent strains. The obtained BM and bioinsecticidal preparations are highly effective and are low cost. Simultaneous synthesis of two biologically active substances - melanin and insecticidal toxins in a single strain will provide the profitability of their production, as well as future commercial success of these preparations for their application in agriculture.

Entomopathogenes against phytopathogens

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The problems of environmental safety are very acute, especially in the field of protecting crops and forests from pests. Most biological products for plant protection are based on bacteria of the genus Bacillus, which exhibit entomocid activities, due to toxins production. This study was aimed to screen Bacillus strains on their producing substances inhibiting the growth of phytopathogens. The influence of strains of Bacillus *thuringiensis* (BT), *Brevibacillus laterosporus* (BL), *Lysinibacillus sphaericus* (LS) on the growth of phytopathogens (15 species) have been studied. All strains used in this work have been taken from Culture collection of Microbial Depository Center (SPC "Armbiotechnology" NAS, Armenia). The antagonistic effect of Bacillus cells and cultural liquid was detected on the agar medium containing phytopatogen in the top layer of agar. The transparent area around the drop of cell suspension or liquid culture was measured. According to obtained results the strain BT-2675 inhibits the growth of five from nine *Pectobacterium carotovorum* species, the rest species were inhibited by BL-105-2 and BL-204. The growth of *Pseudomonas syringae strains* was inhibited by BT-251, BT-248 and BL-204, the growth of *Xanthomonas vesicatoria* – by BL-204 and BT 248. The same effect on the growth of phytopathogens demonstrated cultural liquids produced by these *Bacillus* strains. Thus *Bacillus* strains have been revealed producing antimicrobial substances able to inhibit the growth of several phytopathogenic bacteria.

Yeast biotechnology in VIPECO AM

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Today, diverse yeast species are explored for industrial applications, for example, those of *Saccharomyces*, *Pichia*, *Candida*, *Kluyveromyces marxianus*, *Yarrowia lipolytica*, *Phaffia rhodozyma*, etc. Wild, domesticated and genetically modified yeasts are used in biotechnology. VIPECO AM produces biomass, autolysates and extracts of some of these yeasts and will constantly increase the diversity of exploited yeasts as well as products from them. The plans of VIPECO AM in yeast biotechnology are discussed. For realization of some of these plans, we are collaborating with Institute of Biotechnology (Republic of Armenia) and Skryabin Institute of Biochemistry and Physiology of Microorganisms (Russian Federation).

The hexavalent-chromium reduction by the metal-resistant *Bacillus* sp. NA7 strain

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Hexavalent chromium as a strong oxidizing agent produces mutagenic and carcinogenic effects on living organisms, but a number of bacteria can multiply and grow in heavy metals, including chromates rich in ecosystems.

Therefore, the goal of this work is to isolate the aerobic, endospore generating, chromate-resistant NA7 bacteria isolated from the Artsvanik tailing dump sample, and determine the ability of Cr(VI) recovery.

Microbial identification was carried out by sequencing 16S RNA, according to which the NA7 culture was identified using the type of *Bacillus* sp.

The stability of the strain *Bacillus* sp. NA7 was demonstrated at high concentrations of Cr(VI). The strain *Bacillus* sp. NA7 CrO_4^{2-} MIC is 370 mM, and in the case of $Cr_2O_7^{2-}$ ions 3 mM.

Chromato reductase activity of bacteria was studied colorimetrically at pH 6-11 and at different stages of growth under optimal conditions. It was shown that the maximum chromate reductase activity (81%) of the strain *Bacillus* sp. NA7 appeared at pH 9.0. The growth of the *Bacillus* sp. NA7 strain was inhibited at a concentration of 2.5 mM Cr (VI), but in parallel with the growth, chromate reductase activity increased.

The results show that the strain *Bacillus* sp. NA7 can be used for bioremediation in chromate infected medium.

The developing novel microbial biotechnologies for improving agriculture production in drylands

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Plants are in constant contact with microorganisms. Parasitic, neutral and mutualistic interactions of microorganisms with host plants largely determine their location on different parts of plants: rhizosphere; rhizoplane, phyllosphere and endophytic. The microbiome of rhizosphere and rhizoplane especially their adaptation to other stress conditions of the environment play a key role in host plant-microbe interactions which are largely used in developing microbial biofertilizers for improving agriculture production. Metabolic processes of this microbiome may be a barrier to germination and growth of other pathogenic microorganisms which cause massively fungal and bacterial diseases of plants. Our objective was to develop a new and universal complex microbial biofertilizer on the basis of local strains of microbial consortia. For achieving this purpose, we screened and isolated some plant growth-promoting rhizobacteria from salt-affected soils of Uzbekistan. The isolating, purifying and studying physiologo-biochemical properties, biological nitrogen fixation, ACC and siderophore production traits were performed according to standard methods. The isolated three strains were identified as *Azotobacter chrococcum* A-2, *Bacillus subtilis* SKB-256 and *Pseudomonas putida* SKB-251 and involved in some main traits such as biocontrol for pathogen fungi *Fusarium*, participated in fixing biological nitrogen, produced siderophore and ACC-deaminase and IAA which are directly considered as plant growth-promoting factors. On the basis of these beneficial *Azotobacter chrococcum* A-2, *Bacillus subtilis* SKB-256 and *Pseudomonas putida* SKB-251 strains we have developed a complex biological fertilizer composition for agricultural plants in dryland regions. We suggest that this complex microbiological fertilizer is an effective and ecologically pure fertilizer for developing the organic agriculture in drylands where the fertility and health of soils are affected by several abiotic and biotic causes.

Quantitative determination of inulin with colorimetric reaction in Jerusalem artichoke tubers (*Helianthus tuberosus*)

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Polyfructans are carbohydrate polymers of plant origin, the monomers of which are mainly fructose. The ratio of fructose and glucose in polyfructans is approximately 60:2. They are produced by representatives of various plants, mainly belonging to the families of *Liliaceae*, *Asteraceae*, *Campanulaceae*, etc. [1]. A typical example is inulin from tubers of *Jerusalem artichoke*, which is also known as topinambur. It is a herbaceous plant that forms tubers, containing about 10-15% of inulin [2, 6]. Due to the high content of inulin, the tubers are processed in different countries of the world to obtain inulin-containing powders recommended for the treatment and prevention of diabetes memellitus, atherosclerosis, dysbacteriosis, etc. [2, 3]. The aim of this study is to pretreat inulin-containing raw materials from Jerusalem artichoke tubers, as well as to develop a quantitative determination of inulin by spectrophotometric method with colorimetric reaction [5]. We used standard solutions of inulin, fructose, as well as an inulin-containing extract. To determine the quantitative content of polyfructans in plant materials, optimal extraction parameters were developed: the ratio of raw materials and extractant 1: 2 when heated to 70-80 °C, discoloration of the obtained extract with activated carbon and centrifugation at 8000 rpm. The method of quantitative determination of inulin in terms of fructose was developed using resorcinol and hydrochloric acid [4]. It was determined that inulin-type polyfructans are the least stable class, they undergo hydrolysis in the presence of 1 M hydrochloric acid when heated to 100 °C, as a result of which fructose easily forms a red color complex with resorcinol having a maximum absorption in the range of 440-480 nm. It was shown that this reaction is a highly specific reagent for the determination of polyfructans.

Keywords: polyfructans, inulin, fructose, Jerusalem artichoke, extract

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Creation of new recombinant strain-producers of L-arginine using shuttle expression vector pEC-XK99E

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The development of recombinant DNA technologies allowed to create producers of many biologically active compounds, including amino acids. The increased need for amino acids, associated with the appearance of new areas of their application, required further improvement of producers. This problem can be solved using the methodology of genetic engineering, which allows to increase the expression of the desired gene by multiplying it on multi-copy vectors. Shuttle vectors – bireplicon plasmids that are able to replicate in the cells of various bacteria, were widely used. In this work we used *Escherichia coli–Corynebacterium glutamicum* shuttle expression vector pEC-XK99E for molecular cloning of arg genes (heterologous argJ gene of the thermophilic bacterium *Geobacillus stearothermophilus*, homologous genes argG and argH C. glutamicum) in cells of coryneform bacterium *Brevibacterium flavum*, as well as the construction of strain-producers of L-arginine. Vector pEC-XK99E (7.0 kb) contains a polylinker with 13 sites for restriction endonucleases and a strong promoter Ptrc of *E. coli*. To carry out molecular cloning of arg genes, the genomic DNA of bacteria *G. stearothermophilus* and *C. glutamicum* were isolated, which served as a model for amplification of the corresponding genes. The amplified by PCR genes were ligated with the pEC-XK99E vector. Constructed new recombinant plasmids pARGJ, pARGG, pARGH were introduced by electroporation into the obtained by us strain-recipient Br. flavum HK-19A (ile-, D-sers, ArgHxr, TAr), synthesizing 25 g/L of L-arginine. New recombinant strains containing plasmids with arg genes were obtained. The arginine-producing ability of the recombinant strains was determined first by the microbiological method, then by flask fermentation. Created new recombinant strains in optimized fermentation medium showed increased synthesis of L-arginine (Br. flavum HK-19A (pARGJ) –27.8 g/L, Br. flavum HK-19A (pARGG) – 28.2 g/L, Br. flavum HK-19A (pARGH) – 26.7 g/L), compared with the strain-recipient.

Influence of phe65ala point mutation on substrate specificity of *Geobacillus stearothermophilus* D-hydantoinase

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Hydantoinases play an important role in the production of optically pure amino acids. These enzymes hydrolyze 5-monosubstituted D-hydantoins to produce N-carbamoyl D-amino acids, from which optically pure D-amino acids can be produced by application of D-carbamoylases [1]. To create hydantoinase catalysts with desired substrate specificity, it was proposed to customize its active center towards DL-5- (2-methylthioethyl) hydantoin, DL-benzyl hydantoin and 5-(3'-hydroxybenzyl) hydantoin. Docking analysis was performed by means of AutoDockVina software, above mentioned three molecules were used as ligands. The study of interactions between ligands and macromolecule by means of AutoDockTools software package has shown that Phe65Ala mutation may have a positive effect on enzyme's activity. Site-directed mutagenesis was performed using Agilent Technologies kit. The plasmids carrying mutant gene were transformed into the cells of *E. coli* BL21 Star (DE3) strain. To produce necessary biomass, perform enzyme purification and work with the most active enzyme, the positive colonies carrying hyd gene were grown at 37 °C until OD540 ~ 0.5. Induction of hyd gene was performed under the same conditions in the presence of 1 mM IPTG. Partially purified mutant enzyme was used to identify changes in substrate specificity in comparison with wild enzyme. Our measures showed that Phe65Ala mutant had increased activity towards DL-5-(2-methylthioethyl) hydantoin almost three-fold and almost two-fold towards DL-benzyl hydantoin and 5-(3'-hydroxybenzyl) hydantoin. The study conducted showed newly designed mutant D-hydantoinase with widened substrate specificity, which can be used for production of optically pure D-carbamoyl amino acids. This work was supported by the State Committee of Science under RA MES, in the frame of the research project No.16YR-2I068.

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Study of the ph-dependence of porphyrins binding in complexes with ceruloplasmin for photodynamic therapy of tumors

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Ceruloplasmin (CP) actively binds photosensitizers (PS) and possibly transports photosensitizers to the tumor. Compared to normal cells, cancer cells are characterized by higher intracellular pHi (7.12–7.65) and lower extracellular pHe (6.2–6.9). Changes of pH in the tumor tissue and in its cells can cause modifications in the complexes of CP+PS and change the photodynamic process of tumor destruction.

The aim of this study was to study the complexation of CP with various photosensitizers with a change in pH from neutral to acidic *in vitro*.

Using the methods of absorption and fluorescence spectroscopy in the pH range from 7.1 to 6.1, we studied the binding of ceruloplasmin with cationic porphyrins and metalloporphyrins: TOEt4PyP, Zn-TOEt4PyP, TBut4PyP and Zn-TBut4PyP, as well as neutral Al-phthalocyanine and anionic porphyrin Chlorin e6 (Chl e6).

The absorption spectra showed that for complexes of ceruloplasmin with cationic porphyrins (CP+TOEt4PyP, CP+Zn-TOEt4PyP, CP+TBut4PyP and CP+Zn-TBut4PyP), from pH 7.1 to 6.1 for the Soret band a strong peak shift occurred (from 1 up to 8 nm) and a significant decrease in porphyrin absorption, which indicates a significant change in the microenvironment of porphyrin, as well as significant conformational changes in the protein. Fluorescence studies showed that with decreasing pH, there was an increase in the bound cationic porphyrins on the surface of the protein, which also indicates conformational changes in the protein and the emergence of negatively charged amino acid residues on the surface. Chl e6 is almost completely located inside the protein globule and a small amount is located on the surface.

At the pH changes significant conformational changes of the protein occur, but the photosensitizers do not separate from the protein. The complexes of CP+PS can be carriers of photosensitizers in the blood, but not active agents for PDT of tumors.

Asymmetric synthesis and biological activity of some unsaturated α-amino acids

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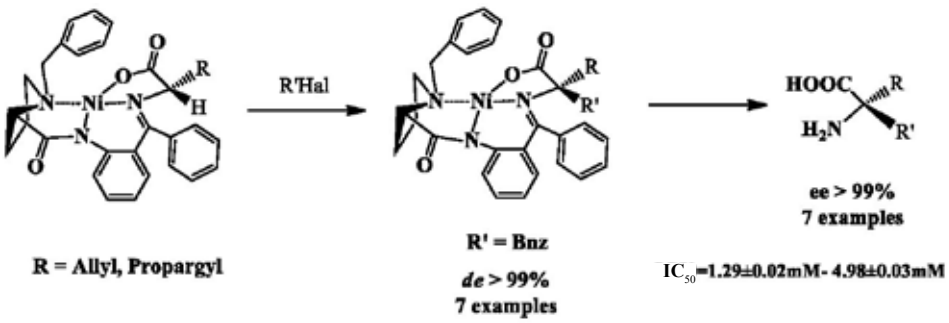
Enantiomerically enriched non-proteinogenic amino acids have been obtained. As a starting amino acid synthon for the asymmetric synthesis of non-proteinogenic unsaturated amino acids, Ni(II) square-planar complexes of Schiff's bases of propargylglycine, allylglycine and glycine with chiral auxiliary (S)-2-N-(N`-benzylprolyl)aminobenzophenone ((S)-BPB) were taken[1,2]. The reaction of alkylation to the C-C bond of propargylglycine, allylglycine and glycine moieties resulted in the asymmetric synthesis of novel (S)-α-propargylglycine, (S)-α-allylglycine and glycine acids containing an aromatic side chain (de 63–95,5%). After purification and cleavage of the metal complexes, the amino acids were isolated with high enantiomeric purity (ee >99%).



Of the obtained 7 non-proteinogenic amino acids, 4 showed inhibitory activity to collagenase G[3]. The amino acid with an acetylene bond in the side chain (IC_{50}=1.29±0.02mM) had the best result. Molecular docking showed that in the amino acids with activity to collagenase G the presence of hydrogen and π-π bonds with the enzyme was observed.

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Biosynthesis of extracellular glucose oxidase by *Penicillium chrysogenum*

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Glucose oxidase (GOX) (β-D-glucose: O_2-1-hydroxy reductase, EC 1.1.3.4) catalyzes the irreversible oxidation of D-glucose to δ-gluconolactone and the concomitant reduction of molecular oxygen to hydrogen peroxide. β-D-glucose $+O_2 \rightarrow$ δ-gluconolactone + H_2O_2. The catalytic properties of glucose oxidase underlie the enzymatic method for determining glucose in biological fluids. The enzyme has been used successfully as the basis for glucose sensors, in kits for determining glucose levels, and as a source of hydrogen peroxide in food preservation. Glucose oxidase is a commercially important analyte with high specificity and stability. These and other beneficial properties have made it the most widely used biosensor enzyme. The main industrial producers of GOX are fungi of the *Aspergillus* and *Penicillium* genera [1, 2]. The great demand for glucose oxidase and its practical value dictates the need for a constant search and selection of new highly active producers of the enzyme and studies to optimize culture media and cultivation conditions.

To obtain seed for growing mycelial fungus *Penicillium chrysogenum*, we have selected the best substrates from wheat, barley, oats, spelt and treated wheat. The maximum formation of GOX under conditions of flask fermentation was noted in experiments using spore seeds obtained on barley and spelt on the 14th day of cultivation and wheat on the 17th day of cultivation.

The effect of carbon-containing components of the nutrient medium: glucose, sucrose, arabinose, maltose, galactose, starch, as well as the influence of biosynthesis effectors of extracellular glucose oxidase of *P. chrysogenum* on the yield of enzyme were studied. It was found that *P. chrysogenum* utilized all tested compounds and in all variants of the experiment the fungus synthesized catalase. Glucose oxidase is the most active when grown on a medium with galactose, the activity is slightly lower on glucose-containing medium, and the least - on the sucrose-containing medium. The influence of effectors on glucose oxidase synthesis, of polysaccharides and alcohols on the synthesis of the target enzyme was revealed [3, 4]. It has been established that the addition of inulin, starch, ethanol, glycine and ethylene glycol to the nutrient medium makes it possible to increase the level of glucose oxidase formation by 70–88% and that of butanol by 7% and alginate by 29%. The use of gelatin and statin in the medium leads to inhibition of enzyme synthesis.

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Possibility of using thermophilic L-aminoacylases of bacilli origin in the asymmetric synthesis of amino acids

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Strains of *Bacillus brevis* MDC 4001, *Bacillus stearothermophilus* MDC 4001 and *Bacillus circulans* MDC 4022 were obtained from Microbial Depository Center of SPC "Armbiotechnology" for testing in the asymmetric synthesis of protein and non- protein amino acids.

When the strains of *Bacillus brevis*, *Bacillus stearothermophilus* and *Bacillus circulans* grown in 100 ml of fermentation broth (0.5% casein enzymatic hydrolysate "Sigma", 0.3% yeast extract "Sigma", 0.2% N-Acetyl-DL-Alanine, 0.3% KH_2PO_4, 0.05% $MgSO_4$, pH 7.2) were filled in 500 ml fermentation flasks and incubated for 20 h at 56 ^oC in rotary shaker at 180 rpm, the specific enzymatic activities was 0.082, 0.118 and 0.091 u/mg, respectively. Disintegration was done by sonication during 20 min in buffer of the following composition: 20 mM HEPES, 100 mM NaCl, 0.05 mM PMSF, 0.1 mM $ZnSO_4$, 1 mM Dithiothreitol, 5% glycerol.

Enzyme preparations were concentrated by "Millipore" 10 kDa MWCO centrifuge ultrafilters, glycerol was added up to 50% and stored at a temperature of -20 ^oC up to usage.

Racemic allylglycine, propargylglycine and alpha-methyl-phenylalanine were synthesized, acetylated (VGh), and tested in 200 mM concentrations, together with N-acetyl-DL-methionine, in deacetylation reactions, carried out by mentioned enzyme preparations. The quantities of the obtained free amino acids after 22 h incubation at 56 ^oC are presented in Table 1.

Table 1

Deacetylation of racemic acyl derivatives of amino acids by crude enzyme preparations

Source of enzyme	Acylated amino acid	Free amino acid, mM
Bacillus brevis	N-acetyl-DL-methionine	18.9
	N-acetyl-DL-allylglycine	25.9
	N-acetyl-DL-propargylglycine	12.1
	N-acetyl-DL-alfa-methyl-phenylalanine	2.4
Bacillus stearothermophilus	N-acetyl-DL-methionine	15.2
	N-acetyl-DL-allylglycine	18.9
	N-acetyl-DL-propargylglycine	13.8
	N-acetyl-DL-alfa-methyl-phenylalanine	2.8
Bacillus circulans	N-acetyl-DL-methionine	59.3
	N-acetyl-DL-allylglycine	88.1
	N-acetyl-DL-propargylglycine	29.6
	N-acetyl-DL-alfa-methyl-phenylalanine	5.9

In used conditions *Bacillus circulans* MDC 4022 exhibited the best catalytic characteristics, so it was chosen for future studies (purification, characterization, immobilisation and catalyst characterization.

New D-aminoacylase strain from the *Rhodococcus* genus

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The 16S rDNA partial sequence of the strain 15.3 isolated from agricultural soil samples from North-East of the Republic of Armenia and carrying the D-aminoacylase activity, revealed its affiliation to genus *Rhodococcus*. We named the strain *Rhodococcus armeniensis* AM15.3. The results of BLAST analyses of partial nucleotide sequence with database of 16S ribosomal RNA gene sequences of Bacteria and Arcahaea revealed maximum of 99.86% identity with 0 gaps and high degree of coverage among 100 BLAST hits. The results for the first 5 BLAST hits are presented in Table 1.

Table 1. The homology between N15.3 strain's 16S rRNA partial nucleotide sequence and 5 BLAST hits of Bacterial and Archaeal 16S rRNA database

N	Source of 16S ribosomal RNA	Identities	Gaps	NCBI Reference
1	Rhodococcus qingshengii strain djl-6-2	1405/1407(99.86%)	0	NR_115708.1
2	Rhodococcus qingshengii JCM 15477 strain djl-6	1405/1407(99.86%)	0	NR_043535.1
3	Rhodococcus degradans strain CCM 4446	1405/1407(99.86%)	0	NR_145886.1
4	Rhodococcus erythropolis strain N11	1398/1408(99.29%)	2	NR_037024.1
5	Nocardia coeliaca strain DSM 44595	1398/1408(99.29%)	2	NR_104776.1

For isolation, purification and characterization of D-aminoacylase of *Rhodococcus armeniensis* AM15.3 the bacterial cells were grown in the medium (pH 7.2) containing 0.5% N-Acetyl-DL-Alanine, 0.3% yeast extract "Sigma", 0.3% KH_2PO_4 and 0.05% $MgSO_4$. Fermentation was done in 500 ml flasks filled with 50 ml fermentation broth and incubated during 20 h at temperature 30 ^oC, in rotary shaker at 180 rpm. Disintegration was done by sonication during 20 min in buffer of the following composition: 20 mM HEPES, 100 mM NaCl, 0.05 mM PMSF, 0.1 mM $ZnSO_4$, 1 mM Dithiothreitol, 5% glycerol.

As a result of chromatographic purification of obtained enzyme preparation on Toyopearl SuperQ-650M "Tosoh" and hydroxyapatite "Sigma" columns, the activity of enzyme preparation increased more than 30 times, reaching 1 u/mg, with near 97% overall yield. Enzyme preparation was concentrated by "Millipore" 10 kDa MWCO centrifuge ultrafilters, glycerol was added up to 50 % and stored at temperature -20^oC up to usage.

Testing of obtained enzyme preparation revealed the mix of L-aminoacylase in D-aminoacylase preparation. Comparison of the activity of preparation with substrate pairs N-acetyl-L-methionine – N-acetyl-D-methionine and N-acetyl-L-valine – N-acetyl-D-valine indicated that the activity of L-aminoacylase exceeds the activity of D-aminoacylase by more than two times. The possibility of liberation of free amino acids from N-acetyl-DL-methionine, N-acetyl-DL-allylglycine, N-acetyl-DL-propargylglycine and N-acetyl-DL-1-methyl-phenylalanine in the presence of mentioned enzyme preparation was shown.

Future purification of mentioned preparation is needed for better characterization of studied perspective catalyst, which can be used as the source of D- and L-aminoacylase.

New α-amylase with potential for commercialization

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Industrial enzyme market has been projected to reach US$ 6.2 billion by 2020. Among major industrial enzymes that find applications in baking, alcohol, detergent, and textile industries are α-amylases. These are produced by a variety of microbes, which randomly cleave α-1,4-glycosidic linkages in starch leading to the formation of limit dextrins. α-Amylases from different microbial sources vary in their properties, thus, suit specific applications. Since α-amylases have a very wide spectrum of applications, there is an increase in the demand for novel α-amylases that have activity and stability characteristics for the industrial harsh conditions.

We demonstrated that the α-amylase from *Bacillus sp.* MDC3500 would have industrial application based on its high specificity to native potato starch cleaving it mostly to glucose and maltose. The protein contains 660 amino acids with 72.39 kDa molecular weight. According to the domain analyses results the enzyme has signal peptide, catalytic domain and carbohydrate-binding module containing 26, 564 and 73 amino acids, respectively. The function of carbohydrate-binding module is to bind starch and it is varying among different α-amylases. The ORF of α-amylase has been cloned and characterized. Noteworthy is the substrate specificity of α-amylase, which was studied at 60 °C in the presence of 1% substrates. The enzyme was active on a number of substrates. Apart from soluble starch, the enzyme also cleaved corn starch, rice starch, potato starch, potato starch Zulkowski, amylose and amylopectin from potato. α-Cyclodextrin was not cleaved by the enzyme, while enzyme shows activity to β- and γ-cyclodextrines. Native potato starch is the best substrate for the enzyme. After 20 hours incubation at 50 °C, the end products of potato starch hydrolyses were identified by HPLC. Obtained data indicate that glucose and maltose are the main products after hydrolysis reactions. The enzyme is pH- and thermostable.

Mentioned characteristics make the α-amylase of *Bacillus sp.* MDC 3500 a useful candidate for industrial application.

Bacteriophage preparation to reduce *Salmonella* colonization in poultry

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Salmonella infection is a global problem and has been attributed to be the most important bacterial etiology for enteric infections worldwide. Food animals are the primary reservoir for human non-typhoid Salmonella infections. In fact, several Salmonella strains persistently colonize chickens but without causing any signs of illness. The ceca of chickens are the major sites of Salmonella colonization, and chickens can become asymptomatic carriers of Salmonella. Existence of such pathogens is problematic not only for animal health, but also for human as possible transmission of antibiotic resistant bacteria from animals to humans through the food supply. The development of alternative anti- microbial remedies has become one of the highest priorities of modern medicine and biotechnology. One of such alternatives might be bacteriophages – bacterial viruses that infect and replicate within bacterial cells causing irreversible death. Aim: The aim of this work is to develop bacteriophage-based product that can be used to reduce Salmonella colonization in poultry.

Isolation and identification of *Salmonella* strains: For isolation of *salmonella* strains total 200 samples of poultry meat and eggs were purchased at the farmer markets and supermarkets in Tbilisi area. Bacterial strains were isolated and identified by standard microbiological and biochemical methods. A total of 31 Salmonella strains were isolated. Bacterial strains: For investigation of bacteriophage host range, apart from new isolated salmonella strains 15 clinical *S. typhimurium* strains from the laboratory collection were used. Antimicrobial susceptibility testing: All the *S. typhimurium* isolates were exposed to different antibiotics for their antimicrobial susceptibility and drug resistance pattern determination using disk diffusion assay following the guidelines of clinical and laboratory standard institute. 8 Different antimicrobial agents, most widely used in clinics were used in this study. Bacteriophage host range and selection of the most efficient phage: The phages were investigated for host range specificity and lysis efficiency against *Salmonella* strains. Each strain was inoculated on Tryptic Soy Agar

(TSA) and 10 µl of phages (1×10 4 - 1×10 7 PFU/ml) was dropped over the plate with inoculated culture. The plates then were incubated during 18 hrs at 37 ºC and the presence of plaques was observed. Transmission electron microscopy: Pure phage stocks with titers of ~10*9 PFU/ml were prepared. Sample of each stock was stained negatively with 1% uranyl acetate, and electron microphotographs were taken at various magnifications. Based on the morphology of the phage, all phages were classified into their respective family according to the International Committee on Taxonomy of Viruses.

Isolation and identification of *S.typhimurium* strains: A total of 31 *S. typhimurium* strains were isolated from 200 samples of poultry meat and eggs. The samples were analyzed for the presence of Salmonella using standard biochemical and serologic methods. Antimicrobial susceptibility testing: The study revealed that the majority of salmonella cultures were characterized by high levels of antibiotic resistance. Multiresistance was revealed when strains were resistant to four and more antibiotics.76% of studied Salmonella isolates was resistant to 4 from 7 examined antibiotics. *S. typhimurium* strains revealed high resistance to Tetracycline (80%), Kanamycin (78%), Streptomycin (67%). Actually, all newly isolated strains were characterized with high to moderate level of antibiotic resistance. Selection and study of effective bacteriophages against Salmonella: We determined the range of host cells susceptibility for selected 8 salmonella bacteriophages. 3 out of 8 phages *Sal.phi*13, *Sal.phi*18 and *vB_stm* 21 were characterized by the widest range of activity. These three lytic salmonella phages were selected to compose the phage cocktail. The phages were mixed in the proportion 1:1:1 and lytic activity and host range of each individual phage was compared with that of the phages cocktail. It was observed that the phage cocktail possessed broader host specificity within *S. typhimurium* serotype than each of three phages alone. The cocktail composed of these phages has shown lytic activity toward 65 out of 66 tested *S. typhimurium* strains (98%). Morphological characteristics of selected Salmonella phages: According to electron microscopy studies phages *Sal. phi* 13 and *vB_Stm* 21 referred to Myoviridae family. Phage *Sal. phi* 18- Siphoviridae family.

The obtained results demonstrate efficacy of phage cocktail that can be used to reduce Salmonella colonization in poultry.

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The use of probiotic labs for feedstuff enrichment

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During the recent years in the Scientific Center of Artsakh within the framework of scientific work supported by the State Committee of Science of the Republic of Armenia we have studied the main fodder resources of the Artsakh Republic and the biodiversity of lactic acid bacteria in different regions of Artsakh, in particular, *Ent. faecium*, *Ent. durans*, *L.helveticus*, which can be used as feed additives. Based on the results 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 a new technology for enrichment of feedstuff was developed in accordance with the main nutritional resources of Artsakh and with probiotic lactic acid bacteria *Enterococcus durans* P13, *Enterococcus faecium* KE5, *Lactobacillus helveticus* KG5, *Streptococus lactis* and *Streptococcus thermophillus*.

The study of probiotic properties of lactic acid bacteria isolated from donkey milk

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The study showed that lactic acid bacteria isolated from donkey milk had high probiotic and antibacterial properties. The studies of probiotic properties of LAB isolated from donkey milk showed that they had high adhesive activity (100%), were resistant to bile by 65% (0.2-0.8% concentration), to proteolytic enzymes by 70% (pepsin, trypsin, proteinase K), to pH (2-9) by 60%, to NaCl by 100% and showed 100% activity against antibiotics. The results showed that the selected LAB had high antibacterial activity to Gram-negative *Salmonella typhimurium* G-38 and Gram-positive *Bacillus subtilis* G17-89 bacteria. The results showed that the LAB isolated from donkey milk inhibited the growth of pathogenic bacteria (*Staph. aureus*, *Ps. aeruginosa*, *Pr. mirabilis*, *Klebsiella pneumonia*) by 60-100%.

Molecular genetic identification of some microalgae from the family *Chlorellaceae*

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Microalgae are the large and diverse group of unicellular microorganisms, which are widely spread in various aquatic habitats: from fresh waters to salt waters [1]. They can double the biomass in a relatively short period of time [3]. They contain a significant amount of valuable substances which play an important role in human body.

The classification of microalgae by taxonomic groups is based on a number of morphological features: pigments and chemical composition, structure of cell membranes, the presence of the motor apparatus, nutrient content and reproduction methods. At the same time, microalgae from taxonomically distant groups can combine characteristics common to each other, and different representatives of the same species can exhibit extreme diversity in shape, size, structure, composition and color. In addition, the same species can switch to different reproduction methods depending on environmental conditions. Thus, without genetic analysis the taxonomic classification of cultures can be unreliable and erroneous.

Currently, molecular genetic methods of analysis are one of the successful, fast and cost-effective tools for assessing biodiversity. Sometimes, they are the only possible tool for establishing the species identity of most microalgae.

The laboratory cultures of *Chlorella vulgaris* Pa-001 and *C. pyrenoidosa* Pa-002 selected from various samples of aquatic ecosystems have been analyzed by molecular genetic methods and analysis of rDNA genes. In accordance with modern taxonomy, it was possible to determine the true species identity of green unicellular microalgae as *Parachlorella kessleri* [2] with high biotechnological potential.

Some sequences of rDNA genes, first obtained for a particular study of mentioned species, can help for implementation of taxonomic ordering and studying family ties, as well as in tracking the evolution of these genes.

The studied ribosomal rDNA genes have a high resolution, in contrast to classical methods. Nevertheless, these genes cannot be used as phylogeographic markers for studying intraspecific differences in microalgae for a number of

reasons, in particular, with a pronounced absence of intraspecific differences, as well as due to intragenomic hypervariability.

Keywords:

microalgae, molecular genetic identification, taxonomic status, phylogeny

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Development of the technology for isolating proline from culture liquid

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The process of electromembrane method for desalting L-proline permeate in a five-chamber electrodyalizer has been studied. In the mode of maximum current density the changes in the DM contents, pH, electrical conductivity of solutions in diluat and concentration chambers, as well as voltage in the circuit in the process of permeate desalting depending on the time were determined.

It was established that the electrodialysis method made it possible with a minimum loss of the target product to purify the permeate from mineral ions (the degree of desalting 98.2%) and accompanying basic and acidic amino acids. The specific energy consumption in the process of desalting permeate was 0.425 A/h/kg, and the current efficiency was 59.1%

An approximate calculation has shown that the electrodialysis method for permeate desalination is 3 times cheaper than the existing ion-exchange method.

The process of separating L-proline from the accompanying initial amino acids of the desalted permeate was studied based on the ability of the accompanying initial amino acids to form Schiff bases with 5-sulfosalicylic aldehyde on the porous anion exchanger APA-8P. It is established that in the process of proline separation from the accompanying amino acids, as well as during their elution, 5-sulfosalicylic aldehyde is not washed out from the resin that allows to use the anion exchanger repeatedly without additional regeneration.

The optimal parameters of the process (values of maximum current density during desalting and purifying, forms of the resin and pH of the desalted solution, as well as the yield of proline from the culture liquid and its purity) have been determined.

The developed method unlike existing technologies enables to isolate the accompanying amino acids from the proline culture liquid in the form of a mixture of amino acids, which can be used in various spheres.

Bacteriophage-probiotic Interplay in Human and Animals Body

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Nowadays phages are recognized as great regulators of the bacterial populations in different ecosystems [1, 2]. Bacteriophages, the bacterial viruses, are found everywhere where their hosts exist, including the human and animal ecosystems [3]. Currently its uses as antimicrobial agents are again in active discussion after the revision of unregulated use of antibiotics and antibiotic resistance dissemination in ecosystems [4].

The search for probiotics and the study of properties of various probiotic cultures led to a conclusion that the best positive results showed probiotic bacteria from the genus *Lactobacillus*. *Lactobacilli* represent heterogeneous populations of bacteria differing in biochemical, cultural, and immune characteristics. Various strains of *lactobacilli* render both typical for other lactic bacteria, and specific influence on a host. They have unequal antagonistic activity against human pathogens. However, some probiotics, including also probiotics of *lactobacilli* origin suppress the growth of other representatives of normal microbiota, including obligates. Besides, the side effect of these probiotics: emergence of inflammatory processes, as well as "lactobacilliosis" and sepsis was also described [5]. Taking into account the high use of probiotics the *lactobacillus* phages deserve core attention.

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Evaluation of small intestine damages of whole body single - dose X-ray irradiated Wistar rats

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Previously the effects of probiotic Lactobacillus acidophilus strain INMIA 9602 Er 317/402 and putative probiotic lactobacilli on DNA damages in small intestine of Wistar rats *in vivo* were shown, and the neutral comet assay was suggested as a tool for *in vivo* selection of putative probiotics with DNA-protective activity [1].

The aim of current study was to investigate the DSB damages in small intestine of the whole body 4.5 Gy X-ray irradiated male Wistar rats. The type 0 (undamaged DNA), type 1 (head diameter: 13.18 μm – 18.06 μm) and type 2 (head diameter: 14.15 μm) damaged DNA comets were studied in control and irradiated rats using the neutral comet assay.

Our current investigations have shown that the comets with the head diameter of 18.06μm described for control-untreated and probiotic groups of rats were not typical for the irradiated rats, while the type 1 comets with small head diameter (9.27 to 12.2 μm) were found in irradiated rats groups only.

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The Microbiota and Human Health

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Huge ranges of microorganisms with various traits known as microbiota live on different surfaces of the human body and, interacting with human cells, promote the prevention or progression of the disease. The recent technological advances in genome sequencing, known as Next-Generation Sequencing, as well as the global assessment of the action of microbial communities on the human proteome and metabolome, have opened unprecedented avenues for understanding of the relationships between human body and bacteria, and promising ways for a more effective treatment of infectious diseases, but also cancers and neurodegenerative pathologies, which seemed to be exclusively specific to humans. This conference will focus on recent successes in the study of the gut microbiota, the mechanisms leading to the progression of severe diseases and the development of new concepts for treatment of pathological processes.

The human body during evolution as a species was accompanied by permanent contacts with a variety of microorganisms including pathogenic agents. Robert Koch proved the place of pathogens in the promotion of specific infectious diseases and the postulates proposed played a remarkable role in the fight against offensive infections. With the discovery of antibiotics and the development of therapy of infections caused by gram-positive and gram-negative bacteria, an era of complete protection of mankind from harmful bacteria seemed to come. However, the ability of bacteria to overcome the action of antibiotics in a relatively short time by emerging resistant forms is a challenge in current medical microbiology.

Metagenomics studies of infectious agents have shown the limits of Koch fundamental postulate "one microbe - one disease". It is now established that pathogens can interact with other microbial communities, through horizontal gene transfer or mutual interference at the levels of proteins and metabolites that influence or aggravate the disease process. Indeed, studies of intestinal microbial communities demonstrate that some commensal bacteria can become virulent under the actions of other microorganisms or antibiotics that cause changes in the composition of the gut microbiota.

The gut is the largest immune organ in the human body comprising the largest interface between the host and the external environment, and

therefore, has important influence on immune dysregulation, and dysbiosis in ill patients. Maintaining the integrity of the healthy intestine by understanding the behaviour of the microbiota in pathological versus physiologically normal conditions can be used as to restore homeostasis, immune responses, and clinical recovery. Much research has been performed in the evaluation of the role of dysbiosis in cancer progression. In particular, the gut microbiota can promote carcinogenesis through induction of pro-inflammatory toxins, alterations in signalling pathways or through impairment of antitumor immune responses or production of reactive oxygen and electrophilic species damaging DNA and proteins.

The relationship between gut microbiota and mental health is one of the most intriguing topics in biology. Reduced memory capabilities have been established as an early sign of developing cognitive and neurodegenerative pathologies such as Alzheimer's disease. Recent human metagenomic studies have revealed better mental health association with the gut microbiota's ability to produce particular neurotransmitters. A set of predictive risk factors for memory decline has been established, which may be converted to potential intervention targets for preventing accelerated decline of the memory, and dementia.

The concept of pathobiome, proposed over the past decade, opens the door to unexplored microbial functions in the human body.

Keywords: microbiota, metagenomics, cancer, neurodegenerative diseases

"Blooming" of the microalgae of phytoplankton as an indicator of the instability of Lake Sevan (Armenia) ecosystem

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Microalgae "blooming" have influence on the physico-chemical and biological parameters of water quality. The morphometric changes of Lake Sevan caused instability of ecosystem. As a result of the water level fluctuations (for example the water level of Lake Sevan has been lowered more than 20m and since 2002 the water level has been increased more than 3m), an increase of anthropogenic impact, as well as climatic change leads to an increase of water temperature of the reservoirs which causes unpredictable succession processes and development of monodominant species in phytoplankton community. The domination of the eutrophycator species in the plankton is considered to be a sign of the eutrophication of the reservoir. Intensive algae "blooming" was recorded in summer of 2018, which was caused by the cyanobacteria Anabaena flos-aquae (66 g/m^3). The most thriving of the blue green algae was registered in the coastal zone. In autumn the "blooming" was caused by diatomic algae Melosira granulate (11 g/m^3). During summer "blooming" the surface water temperature was 20-23 °C, the transparency decreased by 3 times compared to this period of the last year and was about 2 m. The amount of phosphates during the period of intense "blooming" of cyanobacteria was 0.08 mg/l, and the amount of nitrate ions was 0.18 mg/l.

Space shooting of the lake has made it possible to estimate the extent of "blooming", the transformation of biomass and areas of the most thriving of algae, which monitoring investigations will allow predict the phytoplankton "blooming" in the early stage.

Microalgae as a source of carotenoids

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Microalgae are unicellular phototrophic microorganisms that possess the ability to take up H_2O and CO_2 and with the aid of sunlight to convert to complex organic compounds that are subsequently kept inside or released from the cell.

It is known that microalgae respond with physiological alterations to the environmental conditions where they grow [1, 2].

Currently, microalgae are considered as a source of renewable energy (biofuel) and other high-value natural products (proteins, lipids (fatty acids), carotenoids).

Carotenoids are pigments located in the light-harvesting complexes of plants and microorganisms that provide photo-protection and light-absorbing functions [3].

Carotenoids are used as antioxidants, precursors of vitamin A biosynthesis, antitumor and anti-cancer treatments, and to protect skin from harmful effects of sunlight, as well as the membrane system of mitochondria, brain and vascular cells from damages. They are widely used in the pharmaceutical, nutraceutical and cosmetic industries [4, 5]. In aquaculture, carotenoids are included in feed to enhance pigmentation, especially during salmon and ornamental fish cultivation, as well as to induce the immune system [6].

Human and animal organisms do not produce carotenoids and they get the necessary amount of carotenoids from food (feed, vegetable, fish meat).

The main factor for carotenoid production from microalgae from the point of view of eventual economic feasibility is the possibility of operating large photobioreactors, able to handle biomass and metabolites to sufficiently high levels [7]. Therefore, it is important to search for effective producers of natural carotenoids, which will be available in terms of market value.

It is known that the fresh-water microalgae, *Haematococcus pluvialis* (*Chlorophyceae*), accumulates the orange-red pigment astaxanthin and other related carotenoids and their esters [8].

The goal of the investigation was to study carotenoid composition of microalgae *Haematococcus pluvialis*, isolated from the Armenian aquatic habitats and to develop optimise conditions for carotenoids biosynthesis.

For study of carotenoids composition, the strain was cultivated in Tamiya's nutrient medium. For stimulation of carotenoid synthesis, after exponential growth phase, the strain was cultivated under the influence of four different stress factors (light, nitrogen starvation, addition of salt and combination of these conditions).

Total carotenoids, chlorophyll a and chlorophyll b were quantified spectrophotometrically [9].

Table 1. Total carotenoids, chlorophyll a and b concentration in dry biomass under four different conditions of cultivation

Carotenoid pigments in *Haematococcus pluvialis*	**Four different conditions**			
	I	II	III	IV
	Increasing intensity of lighting	Without any sources of nitrogen (N_2) in medium	Addition of sodium chloride (10 g/l)	All three conditions together
	Conc. of carotenoids in dry biomass (mg/g)			
Chlorophyll a	8.265502	1.209589	2.83784	5.357157
Chlorophyll b	5.429941	1.558093	0.969744	2.691404
Carotenoids	231.8729	139.8583	412.4632	259.7899

It was established that the maximum amount of total carotenoids was determined under microalgae cultivation conditions with addition of sodium chloride. The highest level of synthesis of chlorophyll a and chlorophyll b was determined to be under the condition with the increased intensity of lighting.

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How to survive in extreme thermal environments: lessons from viruses

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Aquatic ecosystems with temperatures above 80°C represent habitat of DNA viruses with unusual features, which include amazing diversity of virion architectures - many of which have never been observed among DNA viruses in other habitats - and exceptional genetic content [1, 2]. In common to their hosts -hyperthermophilic archaea-the viruses are adapted to their natural environments and highly stable at temperatures exceeding 80°C. Virion structures of several types of these viruses have been reconstructed at near atomic resolution [3-7]. In my talk I will summarize the results of these studies, which provide insights into molecular basis of the remarkable thermostability of hyperthermophilic viruses.

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Extremophiles found in Armenia: their diversity and biotechnology

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Extremophiles have been intensively investigated last decade and many prospective extremophiles have found various industrial applications as producers of extremozymes and unique biomolecules. Different extremophiles have been isolated from extreme environments in various regions of the globe. Despite its small territory, Armenia is rich by ecosystems characterized with extreme conditions, microbial diversity and biotechnological potential of which, still remains unexplored. Thus, numerous geothermal springs of different geotectonic origins, various saline and hypersaline environments, polymetallic mines and solutional (karst) caves are found on the territory of Armenia. Microbial communities thriving in extreme environments in Armenia have been explored following both cultivation-based and culture-independent approaches.

During last decades the phylogenetic diversity of microbial community thriving in geothermal springs and hypersaline environments located on the territory of Armenia has been explored following both cultivation-based and culture-independent approaches. Near full-length 16S rRNA genes clone libraries construction method, PCR-DGGE fingerprinting method, 454 GS FLX pyrosequencing, Illumina HiSeq2500 paired-end sequencing of metagenomics DNA were applied to obtain information about the occurrence of the dominant prokaryotic populations. Several studies have been performed on the description of novel genera, species and strains, characterization of different bio-resources and whole genome analysis of some isolates.

More than 130 bacilli strains were isolated from Armenian geothermal springs and identified as representatives of the genera *Anoxybacillus, Aeribacillus, Anaerobacillus, Bacillus, Brevibacillus, Geobacillus, Paenibacillus, Sporosarcina, Ureibacillus and Thermoactinomyces.* Representatives belonging to the genera *Halobacillus, Piscibacillus, Bacillus, Virgibacillus, Filobacilus, Streptomyces, Haluarcula, Haloarchaeum and Halobacterium* were revealed from salt subterranean deposits and saline soils in Armenian. Bacilli isolates were also screened for their amylolytic, proteolytic and lipolytic activities, and active producers of thermostable

enzymes were selected. The composition and chemical-physical properties of EPSs produced by two geobacilli strains, production of pigments by thermohilic and halophilic isolates were studied as well. Draft genome *Thermus scotoductus* strain K1 and *Anoxibacillus* sp. strain K103 isolated from Karvachar geothermal spring and two Haloarcula sp. strains were sequenced.

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Metal resistant microbes of Armenian mining areas as new means for bioremediation

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Mines, ores and heavy metal rich environments are exceeding the usual lethal limit of heavy metal ions for living organisms. However, some highly adapted bacteria have evolved metal resistance systems to enable them to grow at otherwise lethal concentrations of the heavy metals and have a potential for application in bioremediation of contaminated environments.

The main goals of the presented work were the determination of the heavy metal resistant microbes in the Armenian mines and tailings based on cultivation and molecular-based approaches, selection of the high heavy metal resistant strains and the study of their ability to detoxify and accumulate toxic metal ions.

A total of 40 mesophilic, acidophilic and alkaliphilic metal-tolerant bacteria were isolated from the samples of the Sotck gold mine, Shamlugh, Kapan, Kajaran copper mines, Akhtala and Artsvanik tailings. Based on 16S rDNA sequence analyses the isolates were identified as members of *Arthrobacter, Algoriphagus, Bacillus, Brevibacillus, Comamonas, Geobacillus, Micrococcus, Methylobacterium, Pseudomonas, Rheinheimera, Sinomonas and Stenotrophomonas* genera. Tolerance towards Cu(II), Cd(II), Zn(II), Ni(II), Co(II), Mo(II) and Cr(VI) was studied, and it was found that all strains are highly resistant to Mo(II), Cu(II) and Ni(II), and sensitive to Cd(II) and Zn(II). *Bacillus* strains also exhibited high resistance towards Cr(VI) and showed toxic chromium reduction ability up to 82% of chromium from the growth medium.

The study of the heavy metal bioaccumulation ability of the strains showed that *B. subtilis* AG4, *B. thermoruber* AG1 and *B. megaterium* AA1 could be consider as high bioaccumulators of Cu (around 70%) and Cd (around 90%).

The draft genome of *B. subtilis* AG4 was sequenced using Illumina. Gene prediction identified metal resistance genes encoding copper-translocating P-type ATPase, lead, cadmium, zinc and mercury transporting ATPase, zinc ABC transporter, and cobalt-zinc-cadmium resistance proteins in the genome of *B. subtilis* AG4.

The expression of the *copA* and *czcD* genes in the presence of different concentrations of Cu(II), Zn(II) and Cd(II) of *B. subtilis* AG4 were studied using RT-qPCR. The highest expression of the *copA* genes was observed at 1.0 mM Cu(II), whereas the highest expression of *czcD* was observed at 0.5 mM Zn(II) and 0.05 mM Cd(II).

The newly isolated metal tolerant strains could have potential in biotechnology and bioremediation.

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