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AUTECOLOGY AND TAXONOMY OF BACTERIA ISOLATED FROM EXTREME ENVIRONMENTS

The paper presents the final results of a five-year research (2011–2015) conducted at the Extremophilic Microorganism Biology Department. Extremotolerant microorganisms were isolated from phytocenoses and soils of West Antarctic; subtropical soils (Israel), karst caves and soils of temperate regions (Ukraine, Bulgaria), silt of hypersaline lakes of Crimea (Ukraine) and the Dead Sea (Israel), highland areas of Andes (Ecuador). 10% of them were resistant to toxic metals, 1-chloro-4-nitrobenzene, UV radiation, high salinity, as well as to low and high temperatures. A method of thermodynamic prognosis of microbial interaction with various toxic compounds was developed, providing a theoretical background for designing new environmental biotechnologies. It enabled to determine the conditions for microbial metabolism regulation that provide for complete degradation of synthetic organic compounds and food wastes as well as for production of energy carriers (H_2 , CH_4 , ethanol, solid fuel). The specified approach resulted an isolation of a number of strains that were resistant to the most toxic metals (Hg^{2+} , Cu^{2+} , Ni^{2+} , Fe^{3+} , Fe^{2+} , CrO_4^{2-}) and performed a variety of interactions with metal compounds including mobilization and immobilization, dissolving and sedimentation. Biodiversity of microorganisms in West Antarctica was studied by means of standard microbiological methods as along with phylogenetic and metagenomic analysis. Pyrosequencing of 16S rDNA amplicons of microbial communities in the studied samples revealed representatives of 20 bacterial phyla. Microbiological methods identified cultivable representatives of four phyla (Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes), which included 25 species. The paper presents the results of taxonomic and/or biotechnology studies of specialized groups of extremophiles: iron-reducing bacteria, hydrogen-producing bacteria and obligate methane-oxidizing bacteria.

K e y w o r d s: extremophiles, Antarctica, the Dead Sea, Ecuador, resistance, toxic metals, UV radiation, 1-chloro-4-nitrobenzene, phylogenetic and metagenomic analysis, environmental biotechnology, biohydrogen.

An intensive study of extremophilic microorganisms (extremophiles) has emerged fairly recently and today these bacteria attract high interest due to their unique ability to exist in extreme conditions unsuitable for other organisms. The research strategy aims to identify new extreme environments for isolation of the promising industrial microorganisms. In natural environments extremophiles are affected by a complex of extreme factors such as radiation, desiccation, toxic metals, temperature, etc. The purpose of this work was to explore the biodiversity of extremophiles in natural environments, to determine their taxonomic affiliation and resistance to a complex of extreme abiotic factors, as well as to estimate their biotechnological potential. The research objects included extremophilic isolates from the polar region (phytocenoses and soils of West Antarctic); subtropical regions (soils from Negev desert,

Israel; alpine massif of Andes, Ecuador) and temperate regions (karst caves and soils of Ukraine and Bulgaria); hypersaline habitats (lakes of Crimea, Ukraine, coast of the Dead Sea, Israel). Specific physiological groups of extremophiles were studied as well: metal resistant, iron-reducing bacteria, hydrogen-producing bacteria and obligate methane-oxidizing bacteria and destructors of synthetic organic compounds (1-chloro-4-nitrobenzene, etc).

Concerning the fundamental and applied aspects, the study of metal resistant microorganisms is one of the most important topics nowadays. Firstly, it broadens the understanding and vision of life existence in the presence of toxic metals. Secondly, it provides patterns for metal transformation in biogeochemical cycles. And finally, it allows to develop new approaches for efficient mining and environmental biotechnologies (purification of concentrated polymetallic sewage, etc.). To address these challenges we have developed a theory of thermodynamic prognosis of microbial interaction with toxic metals [14–16].

Thermodynamic prognosis and microbial resistance to toxic metals.

Microorganisms resistant to extremely high concentrations of toxic metals were found to be abundant in the environments of West Antarctica. Microorganisms resistant to toxic metals (100–1000 ppm of Cr(VI), Cu²⁺, Cd²⁺ and 10–50 ppm of Hg²⁺) were detected in the majority of terrestrial environments (soils, mosses, lichens, lake silt, water, humus) in the studied Antarctic regions. Metal resistant microorganisms *Kocuria carniphila*, *Micrococcus luteus*, *Rhodococcus qingshengii*, *Candida tropicalis* (30000 ppm of Cu²⁺, 1500 ppm of Cr(VI), 800 ppm of Ni²⁺, 500 ppm of Co²⁺ and 100 ppm of Hg²⁺) were isolated from natural environments of Israel, Antarctica and Ecuador. [19, 20, 24, 25].

In accordance with the thermodynamic prognosis, microorganisms reduce only those metals that have the standard reduction potential (E_0') within the thermodynamic water stability ($+814 > E_0' > -414$ mV). An important consequence of these theoretical calculations is that a metal is inevitably reduced even when the in the redox potential between a donor system (metabolically active microorganisms) and an acceptor (metal with strong oxidizing properties) is 100 mV. We theoretically substantiated the growth of microorganisms at very high concentrations of toxic metals-oxidizers, for example at 1 M Hg²⁺, CrO₄²⁻ and Cu²⁺ [17].

Furthermore, each of the toxic metals is a stereochemical analogue of an appropriate “macroelement”, i.e. their ionic radii are equal or similar. For example, toxic Cu²⁺ and Hg²⁺ are the stereochemical analogues of such macroelements as Mg²⁺ and Ca²⁺, whereas CrO₄²⁻ is the stereochemical analogue of SO₄²⁻. This undermines that the acceptor and transport microbial systems “get mistaken” which results an accumulation of toxic metal ions along with the macroelements required for metabolism [16].

Microbial resistance to metals and their interaction are determined solely by thermodynamic conditions: by the difference of potentials in the donor-acceptor systems and by the stereochemical analogy of toxic metals and “macroelements”. This implies that the interaction of microorganisms with metals can occur non-specifically. Therefore, it can be carried out by microorganisms of a wide variety of physiological and taxonomic groups, and metal resistant microorganisms should be present in practically all natural and man-made environments.

Experimental data supporting the theoretical assumptions are presented

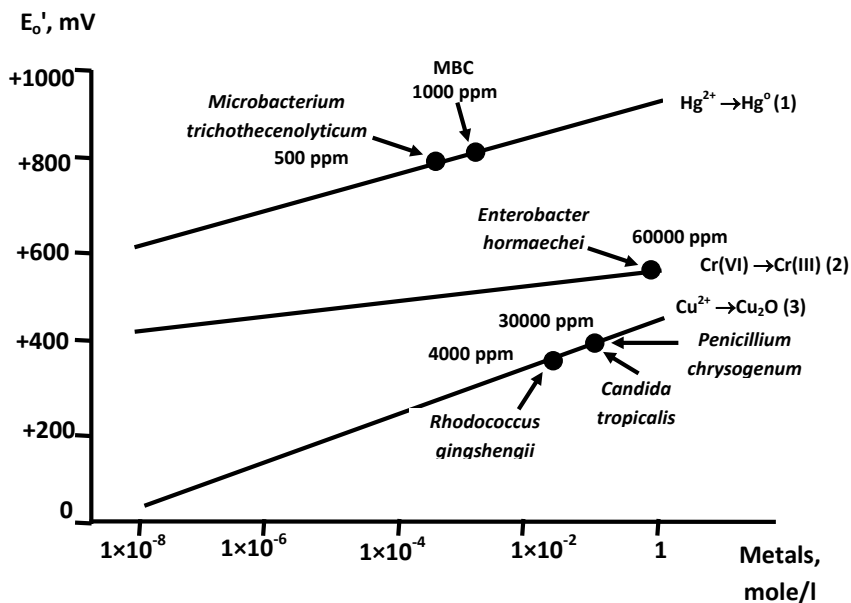


Fig. 1. Experimental confirmation of thermodynamic prognosis of growth of microorganisms* under maximal permissible concentrations of toxic metals

Note: *Mixed microbial communities: *Microbacterium trichothecenolyticum*, *Enterobacter hormaechei*, *Rhodococcus qingshengii* (isolates from Antarctic); *Candida tropicalis*, *Penicillium chrysogenum* (isolates from Ecuador). The reactions shown: (1) $Hg^{2+} + 2e = Hg^0$; (2) $CrO_4^{2-} + (n-1) \cdot H_2O + 5H^+ + 3e = Cr(OH)_3 \cdot n(H_2O)$; (3) $2Cu^{2+} + H_2O + 2e = Cu_2O + 2H^+$.

above. The possibility of microbial growth at extremely high concentrations of the most toxic oxidizing metals (Hg^{2+} , CrO_4^{2-} and Cu^{2+}) is based on the fact that the metal E_o' stays within the zone of thermodynamic stability of water even under multiple increase in the metals concentration (Fig. 1, reaction 1–3). This theoretical position was confirmed experimentally. Thus, microorganisms of the granular microbial biocatalyst MBC (microbial communities of fermented methane tank sludge, etc.) adapt quickly to 1000 ppm of Hg^{2+} [27], antarctic microorganisms grow in the presence of 60000 ppm of Cr(VI) [17], and yeasts from the alpine environment of Ecuador grow at 30000 ppm of Cu^{2+} [6] (Fig. 1).

Thermodynamic prognosis implies not only the resistance of microorganisms to toxic metals, but the inevitable interaction of microorganisms with metals. Microorganisms resistant to oxidizing metals reduce the latter to form insoluble compounds. For example, metabolically active microorganisms from MBC lowered the concentration of Hg^{2+} from 1000 to 0.1 ppm within three days, due to their reduction of Hg^{2+} to Hg^0 through sorption of Hg^{2+} by cells and its deposition by metabolites (Fig. 2).

Ten strains of microorganisms resistant to ultra-high concentrations of Cr(VI) were isolated from pristine Antarctic soils (Fig. 3). These strains were not only highly resistant (up to 30000 ppm) to Cr(VI), but reduced CrO_4^{2-} to insoluble blue-gray $Cr(OH)_3$:



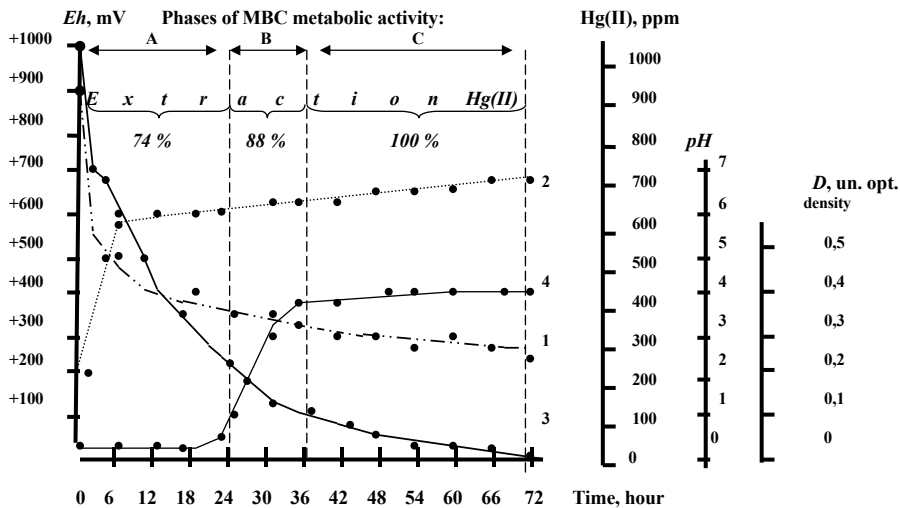


Fig. 2. Extraction of Hg^{2+} by microorganisms of microbial communities of activated sludge granules from model flow in A, B and C phases (weight ratio of microbial communities of granules and solution is 1:40)

Note: 1 – Eh, mV; 2 – pH; 3 – concentration of Hg^{2+} in solution, mg / L; 4 – D, units of optical density (biomass).

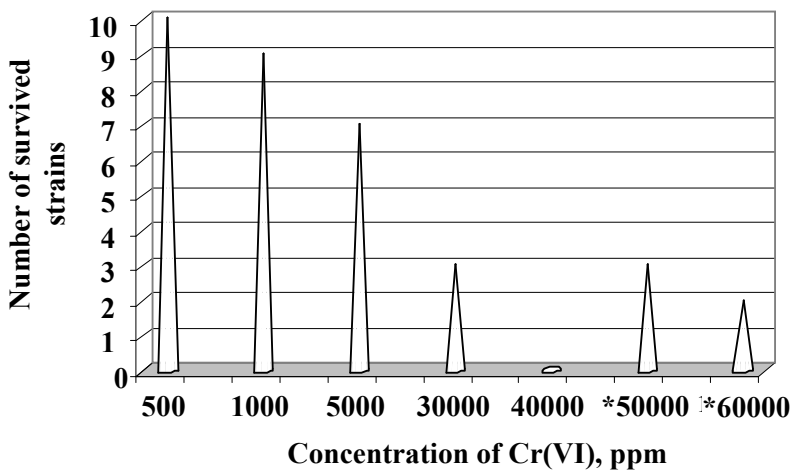


Fig. 3. Resistance of Antarctic strains to ultra-high concentrations of Cr(VI)

Note: *50000–60000 ppm in the presence of electron donor (glucose, 20 g / L of medium).

The resistance to Cr(VI) even increased upon addition of the extra source of carbon and energy (glucose, 20 g/l) to the nutrient medium. Thus, three strains out of ten grew at 50000 ppm of Cr(VI), and two strains grew even at 60000 ppm of Cr(VI) (*Enterobacter hormaechei*) [17]. Similar results were obtained for copper resistant microorganisms from soils of Antarctica and Ecuador. For example, Antarctic microorganisms *Enterobacter hormaechei* and *Brevibacterium antarcticum* resistant to 1000 ppm of Cu^{2+} reduced Cu^{2+} to the insoluble Cu_2O or $CuOH$ in the process of their growth [32]. *Candida*

tropicalis, isolated from the rocky cliffs in Ecuador (4000 m) was resistant to 30000 ppm of Cu²⁺-citrate and provided two opposite processes. It immobilized Cu²⁺ due to its accumulation by the cell biomass (164 mg Cu²⁺ in 1 g of dry cells) and mobilized the insoluble CuO and CuCO₃ by lowering the pH and synthesizing organic acids that chelat Cu(II) (Tab. 1) [6]. These long-term studies confirm the results of our thermodynamic calculations.

Microorganisms resistant to high and ultra-high concentrations of metals were present in all natural environments and constituted 0.01–2.0 % of the total amount of bacteria. The ability of microorganisms to interact with metals proves their significant role in the biogeochemical cycles of metals (migration and redeposition of metals in ecosystems). Thermodynamic prognosis of microbial interaction with metals and its experimental confirmation provide the basis for development of new mining and environmental biotechnologies. For example, galvanic waste water was effectively decontaminated of chromate by microbial communities of activated sludge granules. Metabolically active anaerobic microorganisms of these microbial communities lowered the redox potential to –300...–380 mV, whereas the waste water with 180–200 ppm of CrO₄²⁻ had the redox potential +480 mV. The +780...+860 mV difference in the potentials ensured a rapid decrease in chromium compounds in the solution by means of microbial reduction of CrO₄²⁻ to the insoluble Cr(OH)₃ [21].

Table 1

Metal resistant microorganisms

| Number of strain | Region | Species | Resistance to toxic metals, ppm | | | | | Interaction with metals |
|------------------|-----------|--|---------------------------------|------------------|--------|------------------|------------------|--|
| | | | Cu ²⁺ | Hg ²⁺ | Cr(VI) | Ni ²⁺ | Co ²⁺ | |
| 3201 | Antarctic | <i>Micrococcus luteus</i> | 1000 | 100 | 30000 | – | – | – |
| 3202 | | <i>Enterobacter hormaechei</i> | 1000 | 100 | 60000 | – | – | Reduction of CrO ₄ ²⁻ and Cu ²⁺ |
| 3204 | | <i>Brevibacterium antarcticum</i> | 1000 | 100 | 30000 | 2000 | – | Reduction of CrO ₄ ²⁻ and Cu ²⁺ |
| 3208 | | <i>Microbacterium trichothecenolyticum</i> | 1000 | 500 | 1000 | 2000 | 1000 | – |
| 190n2 | | <i>Rothia nasimurium</i> | 1250 | 50 | 20000 | 2000 | 100 | – |
| KG5 | | <i>Rhodococcus gingshengii</i> | 4000 | 30 | 100 | 100 | 500 | Accumulation Cu ²⁺ in cells |
| PD1 | Ukraine | <i>Rhodotorula</i> sp. | 700 | – | – | – | – | Accumulation Cu ²⁺ in cells |
| H8 | Israel | <i>Micrococcus luteus</i> | 200 | – | 900 | 200 | 200 | Accumulation Cu ²⁺ in cells |
| H7 | | <i>Kocuria carniphila</i> | 200 | – | 800 | 500 | 200 | Accumulation Cu ²⁺ in cells |
| RomCu5 | Ecuador | <i>Candida tropicalis</i> | 30000 | 50 | 1000 | 100 | 50 | Accumulation Cu ²⁺ in cells |
| | | <i>Penicillium chrysogenum</i> | 30000 | – | – | – | – | – |

Note: «–» – was not studied.

Two mechanisms were used for elimination of Cu²⁺ from waste water by an Antarctic copper resistant strain *Brevibacterium antarcticum* in a model system.

The first one is the reduction of Cu^{2+} to the insoluble Cu_2O , and the second one is the accumulation of Cu^{2+} in cells due to the stereochemical analogy of Cu^{2+} and Mg^{2+} . The strain cells immobilized on potato cubes decreased the concentration of Cu^{2+} in the flow bioreactor from 325 ppm to 0.3 ppm [32].

Biotechnology for purification of liquid radioactive waste (LRW) of a broad spectrum of radionuclides (^{51}Cr , ^{54}Mn , ^{60}Co , ^{83}Rb , ^{90}Sr , ^{134}Cs , ^{137}Cs , ^{140}La , ^{144}Ce , ^{226}Ra , ^{238}U , ^{239}Pu , ^{241}Am) was designed based on the stereochemical analogy of radionuclides and macroelements and was tested in a pilot flow bioreactor. The activity of LRW decreased on average by 3–4 orders of magnitude, except for ^{51}Cr , ^{83}Rb and ^{90}Sr , which activity decreased by 6 orders [26].

Thus, the theory of thermodynamic prognosis of microbial interaction with metals is an effective tool for isolation of microorganisms super resistant to toxic metals, for implementation of all known types of microbial interaction with metals and for development of new mining and environmental biotechnologies for industrial wastewater treatment from a wide range of metals and radionuclides.

West Antarctica: taxonomy, autecology and metagenomic analysis of bacteria. Phylogenetic and phenotypic analysis of isolates from ornithogenic soils, vascular plants, mosses, black and orange lichens and biofilm foulings on vertical rocks allowed us to determine primarily the representatives of gram-positive bacteria (*Actinobacteria* and *Firmicutes*). Among *Actinobacteria* there were found *Micrococcus luteus*, *Microbacterium trichothecenolyticum*, *M. phyllosphaerae*, *Rhodococcus fascians*, *Fron dih abitans* spp. and *Arthrobacter* sp., among *Firmicutes* *Sporosarcina aquimarina* and *Staphylococcus* sp. were identified. Gram-negative bacteria are represented by *Pseudomonas mandelii*, *Ps. fluorescens*, *Stenotrophomonas rhizophila*, *Brevundimonas vesicularis*, *Serratia* sp. (*Proteobacteria*), and *Sphingobacterium anhuiense* (*Bacteroidetes*) [9, 10, 11, 33]. This suggests that the terrestrial Antarctic ecosystems are characterized taxonomically diverse bacteria.

All the tested bacteria are capable of growing both at low (1–5 °C) and moderate (18–30 °C) temperatures, regardless of the temperature during the primary isolation of strains from natural sources (1–5 °C or 30 °C). Hence, they are psychrotolerant bacteria [11]. Consequently, the Antarctic strains are able to grow in the temperature range that is representative for the Antarctic. Thus, the temperature from 1 to 5 °C is typical of the polar summer, and on the south side of rocks it reaches 20°C in summer. The destruction of the ozone layer over Antarctica, results in high levels of solar radiation in the region. Therefore, we explored the level of resistance of Antarctic bacteria to UV radiation. It was established that 10–30 % of the isolates from the same sample are resistant to UV radiation (200–500 J / m²) [10, 12].

We studied the structure and diversity of bacterial communities from five soil samples collected on the Fildes Peninsula (King George Island, Antarctica) by using high-throughput pyrosequencing-based analysis of amplified 16S rRNA genes. At the phylum level, the highly diverse soil microbial communities were represented by 19–21 bacterial phyla. The most abundant phylotypes at the genus level in all samples were *Gaiella*, *Gemmatimonas*, *Serratia*, *Spartobacteria*, as well as groups 4, 6, and 7 of *Acidobacteria*. The distance from the coastline and the thickness of the plant cover were found to be

important biogeographic factors influencing the composition of soil microbial communities. Soils from the top of the hills with poor vegetation showed the greatest bacterial diversity – 851 OTUs with the 3 % evolutionary distance, the Shannon diversity index concluded 5.93. Also, fifteen unique genera specific only for these samples were detected. The lowest bacterial diversity (497 OTUs) and the most equable proportion of the dominating phyla were detected in the sample of the coastal soil with thick moss and *D. antarctica* cover. Among all samples, the abundance of *Proteobacteria* was the lowest (20.4 %) but the proportions of *Acidobacteria* (24 %) and *Verrucomicrobia* (16 %) were 2.6–3.6 times higher compared to the soils from the area distant from the coastline. The coastal soil was the only sample that revealed *Gemmatimonadetes* as the dominant phylum (8.5 %), which indicates that the marine environment has a significant impact on the formation of terrestrial microbial communities.

The same soil samples were used to study the formation of metal-resistant microbial communities and their succession in the presence of 200 mg/L of Cu(II) ions during 6 days by means of 454 pyrosequencing of V4–V8 variable regions of 16S rDNA amplicons. Metal-resistant communities included 11 phyla (3–8 phyla in each community, which make up 7–25 % of diversity in the natural samples) – *Proteobacteria*, *Firmicutes*, *Verrucomicrobia*, *Actinobacteria*, *Gemmatimonadetes*, *Acidobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Planctomycetes* *Chloroflexi* and *Nitrospira*. The dominant phylotypes of the Cu-resistant communities – *Pseudomonas*, *Ralstonia*, *Microbacterium*, *Caulobacter*, *Methylobacterium*, *Sphingomonas* and *Bacillus* – were detected in the natural soil samples only as low-abundant or unique sequences. We observed 3 different scenarios of succession in the Antarctic Cu-resistant bacterial communities: 1) rapid increase in the proportion followed by a complete dominance of *Gammaproteobacteria*; 2) shift of the dominant phylum from *Proteobacteria* to *Firmicutes*; 3) gradual increase in the proportion of *Actinobacteria* during the community incubation. The diversity of Cu-resistant community significantly varied and did not depend on the initial diversity of the soil sample. During the microbial community growth and succession, the tendency to diversity decrease was observed. At the same time, at the late stages a surprisingly high diversity of Cu-resistant anaerobic bacteria was found. They belonged to the class *Clostridia-Tissierella*, *Clostridium sensu stricto* and *Anaerosporobacter*. Unclassified bacteria of the family *Ruminococcaceae* formed the dominant phylotype that comprised 30 % of total reads. Three abundant strains representing Cu-resistant Antarctic soil bacterial communities were isolated and identified as *Ralstonia pickettii*, *Pseudomonas veronii* and *Microbacterium laevaniformans*.

Karst (limestone) caves. The presence of ecophysiological groups of nitrogen, carbon and sulfur redox cycles (10^2 – 10^8 cells / g of clay) was demonstrated for the microbial communities of Mushkarova Yuma (Western Ukraine) and Kuibyshev (Western Caucasus) caves. Cave microbial communities included bacteria providing the complete nitrogen cycle. Sulfate-reducing microorganisms were not identified in the sulfur cycle as well as cellulolytic, methanogenic and methanotrophic microorganisms were not detected in the carbon cycle. Microorganisms of karst caves were affiliated with *Microbacterium testaceum*, *Rhodococcus erythropolis*, *Arthrobacter oryzae* and *Pseudomonas azotoformans* species. The studied strains grew in

high concentrations of toxic compounds: 1000 mg / L of Cu^{2+} and 300 mg / L of 1-chloro-4-nitrobenzene and in kopio- oligocarbrotrophic conditions [13, 23, 28]. The specified strains were not only resistant to Cu^{2+} and 1-chloro-4-nitrobenzene, but also interacted with these toxic compounds. Thus, microbial cells accumulated 10 % of Cu^{2+} . The efficiency of 1-chloro-4-nitrobenzene transformation depends on the redox potential and its initial concentration. Thus, we demonstrated for the first time that microorganisms of karst caves are highly resistant to organic and inorganic toxic compounds and are able to detoxify them. It follows that microbial communities are capable of quick and effective adaptation to the alien extreme factors.

The Dead Sea coastal ecosystems. *Bacillus licheniformis*, *B. amyloliquefaciens*, *Bacillus* sp., *Staphylococcus hominis* strains were isolated from the vertical cliffs of clay-salt plains and black highly mineralized mud collected near the Dead Sea. All isolates grew at 0–10 % NaCl concentration at 20–50 °C and were resistant to UV radiation. For *Bacillus* sp. the lethal dose of UV radiation ($\text{LD}_{99,99}$) was 1100–1500 J / m^2 . Several *Bacillus* strains had a strong antagonistic effect on conditionally pathogenic *Staphylococcus aureus* and *Candida albicans* test bacteria.

Andes Mountains and mountain jungles (Ecuador). UV resistant microorganisms ($\text{LD}_{99,99}$ 280–500 J / m^2) were isolated from soil and plant samples of mountain ecosystems (Andes Mountains, Papallacta, 4020 m), from the foot of Tungurahua volcano (1.010 meters) and also from the mountain jungle (La Favorita, 1600 m). Isolates from the Tungurahua volcanic ash and sand were found to be the most resistant to UV radiation ($\text{LD}_{99,99}$ 1000–1440 J / m^2) [18]. Overall, 50 % of aerobic chemoorganotrophic bacteria from Ecuadorian ecosystems are resistant to high doses of UV radiation. Bacteria resistant to toxic metals were detected in the mountainous regions of Ecuador. High resistance to mercury (25 mg Hg^{2+} / L) was shown for the isolate from mountain jungle lichen. *Candida tropicalis* and *Penicillium chrysogenum* isolated from Ecuadorian ecosystems (4000 m) exhibited resistance to 30000 mg of Cu^{2+} / L (in Cu^{2+} citrate form). The isolate from rock lichens was resistant to 3000 mg of Cr (VI) / L [20]. The correlation between the resistance to toxic metals and to UV radiation was not found, probably due to their different cell damage / repair mechanisms.

Fe(III)-reducing microorganisms. Systematic screening of ecosystems of six geographic regions (Antarctic, Arctic, Dead and Black Seas, Ukraine, Georgia and Ecuador) demonstrated a broad area of Fe(III)-reducing microorganisms distribution. There were found microorganisms resistant to citrate-soluble Fe(III) at concentrations ranging from 500 to 20000 mg / L (in terms of Fe^{3+} cation). On the basis of thermodynamic calculations it was shown that microorganisms can carry out both mobilization and immobilization of iron compounds, as well as reduction of Fe(III) to Fe(II). For example, we experimentally verified our thermodynamic prognosis using the strain *Citrobacter freundii* MI-31.1/1. The possibility of microbial metabolism regulation for Fe(III) reduction, mobilization and immobilization of iron compounds [1–4] was demonstrated. Our results contribute to the development of a novel environmental water treatment biotechnology for elimination of iron compounds, as well as to the biotechnology aimed at increasing the efficiency of iron extraction from depleted deposits.

Methane-oxidizing bacteria (methanotrophs). The importance of methanotrophs in nature is determined by their unique ability to obligately assimilate only methane, thus they belong to the extremophiles. Methanotrophs represent unique biocatalytic systems, which can utilize methane of biological and geochemical origin. They serve as a kind of biological filter, which prevents an excessive accumulation of methane in the atmosphere. Ukrainian collection of microorganisms (UCM) includes 50 methane-oxidizing strains from different ecosystems of Ukraine isolated by Department of Gas-oxidizing Microorganism Biology (in 2005 it was renamed as the Department of Extremophilic Microorganism Biology) and 17 more strains provided by foreign researchers. This significantly exceeds the number of methanotrophic strains, which is available in the well-known collections. The audit of the collection has shown that it is necessary to clarify the taxonomic position of 22 strains. On the basis of phylogenetic analysis the examined methanotrophs were classified as the family *Methylococcaceae* (the genus *Methylobacter*) and the family *Methylocystaceae* (the genera *Methylocystis* and *Methylosinus*). According to their phenotypic properties and the results of phylogenetic analysis they belong to «*Methylobacter ucrainicus*» (7 strains), *M. marinus* (5 strains), *M. luteus* (1 strain), *Methylosinus trichosporium* (3 strains), *Methylocystis rosea* (2 strains), *M. hirsuta* (1 strain) [7]. Nucleotide sequences of 16S rRNA genes of 22 methanotrophic strains were deposited in the international database GenBank (GenBank accession No LT220828–LT220849).

Draft genome sequences of five methanotrophic strains isolated from terrestrial habitats were obtained [5]. Of these two strains, *Methylobacter whittenburyi* UCM-B-3033 and *M. luteus* UCM-B-3098, earlier were isolated and studied by researchers in the Department of Gas-oxidizing Microorganism Biology (later in 2005 it was renamed as the Department of Extremophilic Microorganism Biology). Draft genome sequences were generated at the DOE Joint Genome Institute (JGI). These investigations were conducted by 22 scientists from 14 countries. Genome statistics and predicted core metabolic pathways (for the strains UCM-B-3033 and UCM-B-3098) demonstrated the presence of membrane-bound methane monooxygenase (pMMO), PQQ-linked methanol dehydrogenase, PQQ-linked methanol and formaldehyde dehydrogenase, formate dehydrogenase, methanopterin-linked C₁ transfer pathway, folate-linked C₁ transfer pathway, partial serine cycle (i.e., no evidence for glyoxylate regeneration pathway is found), dissimilatory pentose phosphate cycle; assimilatory ribulose monophosphate pathway (RuMP), Entner-Doudoroff pathway, Embden-Meyerhof-Parnas pathway, tricarboxylic acid cycle.

Hydrogen-producing bacteria are used for processing (fermentation) of environmentally hazardous solid organic substrates, organic polymers, and for obtaining gaseous energy carrier, which was the ultimate goal of our research. The principal possibility of producing environmentally friendly H₂ (120 liters/ kg dry waste), as well as low-molecular alcohols (198 g / kg) during fermentation of solid food waste was shown. This process was provided by spore-forming bacteria: *Bacillus licheniformis*, *Bacillus atrophaeus*, *Clostridium butyricum* [22].

It was found that the production of hydrogen and stable and effective waste recycling occurs at establishing and maintaining the optimal pH and

redox potential values. Two optimum pH ranges were determined: 5.0–5.5 – for soil and aerobic sludge microbial communities; 3.8–4.0 – for poultry manure microbial community and aboriginal food waste microflora. The optimal redox potential values for all microbial communities constituted 200 ... –300 mV [29, 30, 31]. Based on the data obtained several processes of food waste fermentation using various metabolic pathways of hydrogen-producing bacteria were developed – ethanol fermentation for recycling of the organic fraction of household landfills, butyric acid fermentation for recycling of multicomponent vegetable waste and metabolic shift fermentation (propionic / butyric) for disposal of cooking waste [29]. It was shown that soil can be a source of hydrogen-producing microbial communities for effective recycling of multicomponent food waste. This idea was confirmed at three sites such as tropical soils of Ecuador forests (a mixture of fruit waste), agricultural soils of South Korea (Korean food waste), and meadow soils of Ukraine (vegetable and cooking waste). In all cases, spore-forming hydrogen-producing microbial communities provided the destruction of 70–95 % of solid waste, and yielded up to 120 liters of hydrogen per kg of absolutely dry waste mass [31].

Thus, microorganisms resistant to toxic metals, 1-chloro-4-nitrobenzene, UV radiation, dehydration, low and high temperatures and specific physiological groups of extremophiles (Fe-reducing, hydrogen-producing bacteria and obligate methane-oxidizing bacteria) were isolated from phytocenoses and soils of West Antarctic, sub-tropical soils (Israel), karst caves and soils of Ukraine and Bulgaria, mud of hyper saline lakes of Crimea (Ukraine) and the Dead Sea (Israel), Andes Mountains (Ecuador, more than 4200 m above sea level) and deep waters of the Black sea. This greatly expands the space for the search of extremophilic microorganisms in natural environments and for development of new environmental biotechnologies.

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АУТЕКОЛОГІЯ І ТАКСОНОМІЯ БАКТЕРІЙ, ІЗОЛЬОВАНИХ ІЗ ЕКСТРЕМАЛЬНИХ ЕКОСИСТЕМ

Резюме

Представлені підсумкові результати п'ятирічних досліджень (2011–2015) відділу біології екстремофілних мікроорганізмів. З фітоценозів і ґрунтів Західної Антарктики, субтропічних ґрунтів (Ізраїль), карстових печер і ґрунтів України та Болгарії, мулу гіперсолоних озер Криму (Україна) і узбережжя Мертвого моря (Ізраїль), високогірного масиву Анди (Еквадор) виділено екстремотолерантні мікроорганізми. Серед них до 10 % – мікроорганізми, стійкі до токсичних металів, *n*-нітрохлорбензолу, УФ радіації, високих концентрацій солей, а також до низьких і високих температур. Для створення природоохоронних біотехнологій розроблено метод термодинамічного прогнозу взаємодії мікроорганізмів з ксенобіотиками. Вперше на його основі експериментально визначено умови регуляції мікробного метаболізму, які забезпечують глибоку деструкцію синтетичних органічних сполук

і харчових відходів з одночасним отриманням енергоносіїв (H₂, CH₄, етанол, тверде паливо). Виділено мікроорганізми, стійкі до найбільш токсичних металів (Hg²⁺, Cu²⁺, Ni²⁺, Fe³⁺, Fe²⁺, CrO₄²⁻), а також показана можливість як мобілізації, так і іммобілізації металів. Стандартними мікробіологічними методами, а також за допомогою філогенетичного і метагеномного аналізу показано різноманіття мікроорганізмів у Західній Антарктиці. Піросеквенування ампліконів 16S рДНК мікробних угруповань у досліджених зразках виявило представників 20 бактеріальних філ. Мікробіологічними методами виявлено культивовані представники чотирьох філ (*Actinobacteria*, *Firmicutes*, *Proteobacteria*, *Bacteroidetes*), які включали 25 видів. Наведено результати по таксономії та/або біотехнології спеціалізованих груп екстремофілів: залізвідновлюючих, воденьсинтезуючих і облігатних метаноокислюючих бактерій.

Ключові слова: екстремофіли, Антарктика, Мертве море, Еквадор, стійкість, токсичні метали, УФ радіація, *n*-нітрохлорбензол, філогенетичний і метагеномний аналізи, природоохоронні біотехнології, біоводень.

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АУТЭКОЛОГИЯ И ТАКСОНОМИЯ БАКТЕРИЙ, ИЗОЛИРОВАННЫХ ИЗ ЭКСТРЕМАЛЬНЫХ ЭКОСИСТЕМ

Резюме

Представлены итоговые результаты пятилетних исследований (2011–2015) отдела биологии экстремофильных микроорганизмов. Из фитоценозов и почв Западной Антарктики, субтропических почв (Израиль), карстовых полостей и почв Украины и Болгарии, ила гиперсоленых озер Крыма (Украина) и побережья Мертвого моря (Израиль), высокогорного массива Анды (Эквадор) выделены экстремотолерантные микроорганизмы. Среди них до 10 % – микроорганизмы, устойчивые к токсичным металлам, *n*-нитрохлорбензолу, УФ радиации, высоким концентрациям солей, а также к низким и высоким температурам. Для создания природоохранных биотехнологий разработан метод термодинамического прогноза взаимодействия микроорганизмов с ксенобиотиками. Впервые на его основе экспериментально определены условия регуляции микробного метаболизма, которые обеспечивают глубокую деструкцию синтетических органических соединений и пищевых отходов с одновременным получением энергоносителей (H₂, CH₄, этанол, твердое топливо). Выделены микроорганизмы, устойчивые к наиболее токсичным металлам (Hg²⁺, Cu²⁺, Ni²⁺, Fe³⁺, Fe²⁺, CrO₄²⁻), а также показана возможность как мобилизации, так и иммобилизации металлов. Стандартными микробиологическими методами, а также с помощью филогенетического и метагеномного анализа показано разнообразие микроорганизмов в Западной Антарктике. Пиросеквенирование ампликонов 16S рДНК микробных сообществ в исследованных образцах выявило представителей 20 бактериальных фил. Микробиологическими методами выявлены культивируемые представители четырех фил (*Actinobacteria*, *Firmicutes*, *Proteobacteria*, *Bacteroidetes*), которые включали 25 видов. Приведены

результаты по таксономии и/или биотехнологии специализированных групп экстремофилов: железовосстанавливающих, водородсинтезирующих и облигатных метаноксилирующих бактерий.

Ключевые слова: экстремофилы, Антарктика, Мёртвое море, Эквадор, устойчивость, токсичные металлы, УФ радиация, *n*-нитрохлорбензол, филогенетический и метагеномный анализ, природоохранные биотехнологии, биоводород.

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